**ISBN 1-878736-02-7 Paperback**
**Fourth Edition**

Published by
**The Sprout House, Inc.**
PO Box 1100
Great Barrington, MA 01230

*God Bless*
*Ann Wigmore and Viktoras Kulvinskas,*
*Prophets of Health and Spirit,*
*Whose Teachings*
*Have Given Birth*
*to a*
*New Generation of*
*Healers*

Of course, you can eat these sprouts before they mature, but you would be losing a lot. The popularity of sprouts is based on their reputation as nutritional superfoods. But this is not the case if they are not fully mature! Their nutritional peak usually occurs at the time of their first leaf division. Many restaurants serve a salad full of brown and yellow sprouts. The brown parts are the hulls which have not fallen off yet and the yellow represents the lack of full chlorophyll development. Simply speaking, you are not getting what you are supposed to. Not only that, certain undesirable factors remain present within the seed until the plant fully develops *(see p. 113)*. Once you grow a delicious crop of mature green sprouts, you will never eat them any other way. Eating immature sprouts shortchanges you in total yield as well as nutrition. Patience pays.

## What Seeds To Sprout

Your sprouter is ideal for growing indoor vegetable seeds that develop chlorophyll-rich, green leaves. These include:

| | | |
|---|---|---|
| Alfalfa | Garlic | China Red Pea |
| Clover | Onion | Turnip |
| Fenugreek | Mustard | Cabbage |
| Radish | Buckwheat | Broccoli |
| Kale | Sunflower | Chia |

## Which Seeds - Which Size - How Much

| *6" 2-3 Tbsp* | *8" 5 Tbsp* | *9" 6-7 Tbsp* |
|---|---|---|
| Radish | Alfalfa | Buckwheat |
| Garlic | Clover | Sunflower |
| Onion | Fenugreek | China Red Pea |
| Cabbage | | |
| Kale | | |
| Turnip | | |
| Chia | | |
| Mustard | | |



**6 INCH BASKET, 2 - 3 TABLESPOONS SEED**

These varieties are hot and/or spicy. Use the smallest 6 inch basket for them unless you have a spicy appetite. Use 2-3 tablespoons of seed. Garlic, Onion, Radish, Cabbage, Turnip, Kale, Broccoli, Mustard, Canola, Chia. Garlic and onion are delicious and very hearty. Mustard is hot. Cabbage, turnip, kale, broccoli and canola are all cabbage family. Chia is a gelatinous seed *(see p. 157)*.

**8 INCH BASKET, 5 TABLESPOONS SEED**

Alfalfa, Clover and Fenugreek. Clover is a spicy cousin of alfalfa with bigger leaves. Fenugreek is a bitter herb and very healthy for the respiratory system. Use it mixed with alfalfa for best taste. 5 Tbsp can yield one pound of salad greens

**9 INCH BASKET, 6 - 7 TABLESPOONS SEED**

Buckwheat, Black Skin Sunflower, China Red Pea. These three seeds represent the largest leaves and tallest stalks of the sprouting family. Mung beans may also be grown this way even though they are not a salad green. Choose only *whole* buckwheat and sunflower *in-the-shell*.

## Double Decker Technique

*Stack Your Sprouters!* Since space is often a problem, here's a technique to conserve it. Two sprouting baskets on top of each other take up less space than two side by side. During the first phase of germination (days 1-4), any two of the sprouters could be stacked with the smaller basket underneath the bigger one. Insert the double decker into the greenhouse.

It's a great space saver, but that's not all. Seeds send their roots vertically downward searching for soil. The extra height of the double decker gives the roots from the top basket plenty of room to stretch. Ordinarily, they are matted underneath the basket by the floor of the greenhouse tent. Elevating the basket gives the roots space to breathe and has the potential to increase the length of the stalks.

## ugreek

*ovella foenum graecum*

ugreek is actually a member of the legume (leguminosae) It is a cousin of clover and luceme (alfalfa). The Pharaohs of sed it in religious ceremonies. The monks of the Middle Ages : to treat blood poisoning, failing eyesight, fevers, palpitations er and kidney troubles. It is widely cultivated in Arab coun- here it was traditionally used to stimulate appetite. Its chemi- nposition resembles that of cod-liver oil and is considered a herb' to garlic, enhancing that herb's disinfectant properties is mic because it is so rich in many minerals including id sulfur and vitamin E. It 'feeds' the blood and is recom- l for ailments that are associated with weakness such as ane- l infections. Both the seed and the whole plant are used.

ugreek is a demulcent meaning it is soothing to the mucous anes and reduces inflammations. A tea made from the seed is a gargle and for sore throats. It also acts as an expectorant, ; the mucosa of the chest and respiratory system. Byzantium s used it to increase their milk supply. Poultices made from k and leaves have been used on wounds, boils, sores and . The seed contains beneficial volatile oils and steroidal ns which may be used to regulate blood cholesterol. ek sprouts have both the properties of the seed as well as t. This sprout should be used to stimulate and to fortify.

### Nutrition in Fenugreek Seed

*(in Milligrams per 100 grams)* [30]

| | | | |
|---|---|---|---|
| in | 23.0 | Zinc | 2.50 |
| les | 323.0 | Niacin | 1.64 |
| ım | 176.0 | Iron | 33.53 |
| fat | 6.4 | Arginine | 2.47 |
| sium | 191.0 | Leucine | 1.76 |
| horus | 296.0 | Lysine | 1.68 |
| ium | 770.0 | Aspartic acid | 2.71 |
| n | 67.0 | Glutamic acid | 3.99 |



## Cabbage

*Brassica oleracea*

The cabbage family of foods includes Chinese cabbage, broccoli, kale, turnip, rutabaga, radish, mustard, rape, cauliflower, collard greens, brussel sprouts and kohlrabi. Of these, the first eight are good for home sprouting. Cabbage is rich in fiber and a good source of minerals especially potassium 253mg per 100 grams, sulfur 1710mg and vitamins C 47mg, E and A 200 IU. It has a drying and binding faculty that makes it effective for inflammations and hot swellings. Historically, cabbage was used to combat scurvy at sea even by the famous Captain Cook. Sailors would make sauerkraut from it which coated their intestinal tract with friendly bacteria and promoted regularity. The fermentation from the kraut remedied the complaints of flatulence that are common with the cabbage family. It is also improved by boiling and draining. European literature often mentions cabbage juice as the best medicine for hangovers. Philip Moore in the *Hope of Health* in 1564 wrote, "the juice of cabbage purges the head, being put into the nostules. Being taken after much drinking, it withstandeth drunkenness."

The cabbage family and other cruciferous vegetables are now taken seriously at the National Cancer Institute. Worldwide epidemiological studies consistently point to lower than average cancer rates for those groups regularly eating dark green leafy vegetables. The crucifers contain compounds called glucosinolates which block the development of cancer. Turnip greens contain between 39 and 166 milligrams per hundred grams of glucosinolates. When cooked, the concentration drops to a range of 21-94 [46].

Cabbage has the greatest potential in colon and stomach cancer. Several major epidemiological studies demonstrate that eaters of leafy green crucifers have the lowest rate of colon cancer. Other population surveys add cancers of the prostate, rectum, esophagus, lung and bladder to the list. In May 1978, Lee Wattenberg, M.D., a professor of pathology at the University of Minnesota Medical school, reported in the journal *Cancer Research*, that he had isolated chemicals called indoles from cruciferous vegetables which were potent

. Common black pepper contains nearly 10% (by weight) piperine, .erine is related to safrole which causes cancer in mice. Should therefore deduce that black pepper, a condiment on nearly every ing table in America, causes cancer in humans? Aflatoxin is one the most potent carcinogens known and just hearing its name is rming to the public. It can be a contaminant in moldy bread, ese, corn, peanuts and fruit, but it is extremely rare. osamines and nitroso compounds are suspected causes of stom- and digestive tract cancers. Beets, celery, lettuce, spinach, radish rhubarb all contain 200 milligrams or more of nitrates (per 100 ortion). Should we incriminate these common vegetables, ...ed for thousands of years across multi-national and cultural ders because chemical components isolated within them have onstrated mutagenic effects on rats?

## Anti-Oxidants & Anti-Carcinogens

All right. Nature is not benign. Natural toxins do exist. But natural ds and particularly sprouts, also contain numerous beneficial ymes, anti-oxidants and anti-carcinogens such as vitamin E, beta- otene, selenium, super-oxide dismutase and ascorbic acid (vitamin that act as the body's defense mechanism against toxins whether ral or man-made.

Beta-Carotene is found in mature alfalfa sprouts and in all plants ontain chlorophyll. It is a very efficient free radical trap [17] demonstrated anti-carcinogenic activity in rats and mice [18] nium significantly inhibits skin, liver, colon, and mammary ors in experimental animals by a variety of carcinogens [19]. athiones, rich in foods containing the sulfur amino acids, are r anti-oxidants and anti-mutagens and may even be effective st potent aflatoxins [20]. Vitamin C (ascorbic acid) was shown to nti-carcinogenic in rodents treated with ultraviolet radiation and te. Mushrooms like shitake contain the active polysaccharide pound lentinan. Lentinan stimulates interferon production. feron is a powerful anti-tumor agent [27].



*"We know that "free radicals" are the guilty party because chromosome breaks created in the presence of L-Canavanine sulfate were prevented by the anti-oxidant superoxide dismutase [16]."*

Raw and sprouted vegetables contain enzymes that oppose tumor growth. Tumors release enzymes called proteases which break down healthy tissue around the tumor and increase potential tumor growth. Inhibiting enzymes in live foods called protease inhibitors, block the actions of these proteases and the spread of the tumors. Sprouted seeds and beans, particularly soybeans and lima beans, are our finest dietary sources of these protective enzymes [23].

Flaxseeds and their young sprouts are one of our best dietary sources of the essential omega-3 fatty acids such as alpha-linolenic acid. Freshly sprouted 1-2 day flaxseeds provide an excellent source of this extremely unstable oil. Studies show that the omega fatty acids have an inhibiting effect on tumor growth [24]. Specifically, they decrease the synthesis of prostaglandins thus decreasing the migratory ability of tumor cells and metastasis [25].

Sprouts also show promise to help in the fight against breast cancer. Soybean sprouts are nature's finest source of plant isoflavones which are converted in our stomachs to isoflavone equol. High estrogen levels stimulate breast tumor growth, but research shows isoflavone equol to have excellent anti-estrogenic qualities similar to that of cruciferous vegetables [26].

In 1992, researchers at Johns Hopkins University Medical school isolated sulphoraphane, a compound found in broccoli and other brassica family vegetables. Sulphoraphane stimulates a cell's production of certain protective enzymes that resist tumor growth [9]. Studies of cancer patterns in the U.S. and abroad reveal strong statistical linkage between the consumption of raw vegetables and relative immunity to a variety of cancers. Researchers have long known that cells exposed to carcinogens respond by generating an assortment of highly effective enzymes that guard against malignant growth. They

appear to work by bonding with the toxins and preventing their chemicals from reaching the cell's vulnerable genetic material. Then, they flush them from the body. The most effective enzyme stimulated by the sulphoraphane in cabbage family foods is called quinone reductase. Sulphoraphane, by the way, is related to mustard oil. Foods that contain sulphoraphane are cabbage, broccoli, kale, cauliflower, turnip, Chinese cabbage, collard greens, brussel sprouts and even non-cruciferous vegetables like carrots, green onions, chives and the sprouts of broccoli, kale, turnip, garlic, onion and Chinese cabbage.

Chlorophyll, one of the most basic nutritional elements in plants, is a well known blood purifier and, in fact, is similar in chemical structure to human hemoglobin. Numerous animal studies demonstrate that chlorophyll can be converted into hemoglobin. Alfalfa sprouts are one of our best dietary sources of earth grown chlorophyll. (Algae from lakes is highest.)

Alfalfa sprouts have also demonstrated a remarkable cholesterol reducing capacity. Studies in both humans and a wide selection of animals including dogs, rabbits, chickens, pigeons and pigs have shown a regression of atherosclerosis [40] and a considerable drop in the levels of serum cholesterol. Saponins in alfalfa appear to be responsible for lowering cholesterol and balancing the bile [41]. They create a sudsing action that prevents cholesterol and bile salts from being absorbed. Although there has been concern in the past about the toxicity of saponins, research showed positive results in the lack of toxicity of alfalfa saponins in monkeys and rats [42].

Enzymes are protein-like chemical agents that facilitate all life-building processes such as digestion, absorption and metabolism. The enzyme and anti-oxidant super-oxide dismutase, abundant in sprouts especially green sprouts like alfalfa, obstructs the free radical-canavanine-alfalfa pathology. In a 1980 report published in *Human Genetics*, chromosome breaks caused by free radicals were prevented by the anti-oxidant super-oxide dismutase [16]. In a 1993 study at the Indiana University School of Medicine, 78 female mice



received a lethal dose of 580 rads of x-radiation designed to caus extreme free-radical activity. Half of the 23 placebo-fed mice die within 30 days. The remaining 55 mice were fed supplements mad from wheat sprouts. All of them survived except one. Wheat sprout are high in the pre-cursor enzyme that stimulates the body's manu facture of super-oxide dismutase [35].

Wheat sprouts have also demonstrated anti-mutagenic activity i mice and rats in three separate studies. Members of the flavonoi family, shaftoside and swertisine, both glycosides of apigenin appea responsible for the wheat sprouts' strong anti-mutagenic behavio [38]. The sprouts were not grown to the grass or green stage.

Perhaps because of their rapid germination and protein manufac ture, sprouts are also rich sources of nucleic acids. Nucleic acids ar the genetic keys to protein and tissue growth found in th cytoplasm, nucleus and chromosomes of cells. They resist cell muta tion and promote healthy cell growth. These results indicate tha sprouts have a profound effect on our ability to fend off free-radica induced diseases such as cancer and immune system disorders.

## Now for the Real Carcinogens

Rather than isolating and attacking natural toxins in plants whic are balanced by a multitude of enzymes and nutrients, perhaps w should turn our efforts to eliminating known carcinogens in ou environment. Free oxygen radicals are caused by numerous dietar and lifestyle factors including medical drugs, air and water pollutio pesticides, alcohol, cigarettes, fried foods, smoked and barbecue foods, nitrates, even good old toast and coffee.

Charred meats and rancid fats should not be part of anyone' diet. The heating of proteins and fats creates a variety of DNA dam aging agents [22]. So does the carmelization of sugars and amin acids visible on the browned ends and crust of common toaste bread. In fact, the amount of burnt and browned material in th human diet may be several grams per day. In comparison, a 2 pac

THE GOOD NEWS SPROUTS RECIPE BOOK
*ISGA / August 1992*


Published by the International Sprout Growers Association, 7964 Pocket Rd., #18
Sacramento, CA 95831

*Published simultaneously in the United States and Canada*
PRINTED IN THE UNITED STATES OF AMERICA

# Contents

# Preface

## The Good News

*"The vitamin-boosting power of the Sprouting process is so impressive that it's truly a wonder sprouting is not used much more widely to make grains, beans and other seeds into convenience foods of outstanding nutritional value... (and taste)."*

**Nancy Albright**
NATURALLY GREAT
FOODS COOKBOOK
**Rodale Press**

While the Good News is definitely GOOD, it's not NEW. Sprouting goes back 5,000 years to ancient China where physicians prescribed sprouts as a treatment for a variety of ills. Brought to North America by Asian immigrants, sprouts remained primarily in the Asian diet until the 1960's. The dawning of the "New Age" fostered interest in more natural, fresh and nutritious foods and led the rediscovery of SPROUT POWER.

Today, the Good News is that sprouts are becoming recognized as one of nature's most nutritious, freshest and most economical produce items. The commercial sprout industry produces in excess of 182 million pounds of over 20 varieties of fresh sprouts annually. More Good News is that sprouts are found in over 90% of the retail food stores and supermarkets across North America. Fresh sprouts provide protein, vitamins A, B, and C, and niacin with no fat and few calories for only pennies per serving.

Finally, the really Good News is that the International Sprout Growers Association (ISGA) has published ***THE GOOD NEWS SPROUTS RECIPE BOOK.*** This collection of over 70 nutritious, delicious and fun recipes contributed by members of ISGA, is written to provide consumers with ideas for using sprouts in a variety of breakfast, lunch, dinner, and snack dishes. These recipes feature several sprout varieties and are very easy to prepare.

ISGA hopes you enjoy the dishes in ***THE GOOD NEWS SPROUTS RECIPE BOOK*** and invites you to explore other exciting and delicious ways you can use fresh, tasty, nutritious sprouts in your daily meals.

# Introduction

## Sprout History

Medicinally and nutritionally, sprouts have a long history. It has been written that the Ancient Chinese physicians recognized and prescribed sprouts for curing many disorders over 5,000 years ago. Sprouts have continued to be a main staple in the diets of Americans of Oriental descent. Although accounts of sprouting appear in the Bible in the Book of Daniel, it took centuries for the West to fully realize its nutrition merits.

In the 1700's, sailors were riddled by scurvy (lack of Vitamin C) and suffered heavy casualties during their two to three year voyages. From 1772-1775, Captain James Cook had his sailors eat limes, lemons and varieties of sprouts--all abundant holders of Vitamin C. These plus other fresh fruits and vegetables and a continuous program of growing and eating sprouts were credited with the breakthrough, thus solving the mariners' greatest casualty problem.

During World War I, sprouted lentils were made a part of the diet of British and Indian troops, stationed in Mesopotamia, to cure scurvy.

During World War I and II, the United States prepared for massive protein (meat) shortages (which never occurred) by educating the public regarding alternative protein supplements--SPROUTS! Alfalfa seeds are 35% protein! The U.S. Department of Agriculture and Agriculture Extension Service held seminars, sponsored radio broadcasts and published literature on sprouts. Major magazines such as "Better Homes and Gardens", "Popular Mechanics", "Readers Digest", and "Life" ran articles and recipes on the preparation and use of sprouts in the family diet.

## Nutritional Advantages of Sprouts

It is really only in the past thirty years that "westerners" have become interested in sprouts and sprouting. During World War II considerable interest in sprouts was sparked in the United States by an article written by Dr. Clive M. McKay, Professor of Nutrition at Cornell University. Dr. McKay led off with this dramatic announcement: "Wanted! A vegetable that will grow in any climate, will rival meat in nutritive value, will mature in 3 to 5 days, may be planted any day of the year, will require neither soil nor sunshine, will rival tomatoes in Vitamin C, will be free of waste in preparation and can be cooked with little fuel and as quickly as a ...chop."

Dr. McKay was talking about soybean sprouts. He and a team of nutritionists had spent years researching the amazing properties of sprouted soybeans. They and other researchers at the universities of Pennsylvania and Minnesota, Yale and McGill have found that sprouts retain the B-complex vitamins present in the original seed, and show a big jump in Vitamin A and an almost unbelievable amount of Vitamin C over that present in unsprouted seeds. While some nutritionists point out that this high vitamin content is gained at the expense of some protein loss, the figures are impressive: an average 300 percent increase in Vitamin A and a 500 to 600 percent increase in Vitamin C. As a result, one-half cup of almost any sprouted seed provides as much Vitamin C as six glasses of orange juice. In addition, in the sprouting process starches are converted to simple sugars, thus making sprouts easily digested.


**President, Ms. Sprout, says:**

I couldn't believe it: I had been a home sprouter before going into business supplying sprouts to supermarkets; the sprouts loved growing in our big batches, 80 pounds per tray.

We got better quality, better tasting, longer lasting sprouts. Part of it is that we learned some tricks, but mostly, I think, they really like being crowded together.

And sprouts generally stay fresh in the store because they are still growing, 'though refrigeration slows them down...brrr. But as you know, sometimes they are limp and tired and watery and old, and then we don't want you to buy them and be disappointed. We want you to tell the store personnel that you want fresh, alive sprouts!

# Product Information

Sprouting increases the nutrition value of seeds. Research nutritionists at such respectable schools as Yale, Cornell, McGill and the University of Pennsylvania have found that sprouts retain the B-complex vitamins that are in the originial seeds, and in the process of sprouting, show a big increase in Vitamin A and an enormous increase in Vitamin C; the Vitamin C content of sprouted mung beans is several hundred times more than unsprouted beans. Sprouting also converts starches into simple sugars, making sprouts easy to digest.

## BEANSPROUTS

The term "BEANSPROUTS" is usually associated with the delicate shoots of mung beans. They have small light yellow leaves, a silvery white shoot, and a root that may be slightly darkened.


Beansprouts have a subtle, nutty flavor. They possess a high water content which makes them refreshingly juicy. Grown locally year round, beansprouts are a good source of protein and Vitamin C. A 12 ounce bag of beansprouts served as a side dish or salad is enough for 4 to 6 people.

When purchasing beansprouts, look for firm, white shoots.

Store in the vegetable crisper of your refrigerator. It is best to use beansprouts as soon as possible. Their life can be extended by rinsing daily in cold water and draining before returning to the refrigerator. Beansprouts can be frozen if they are to be used in cooking.

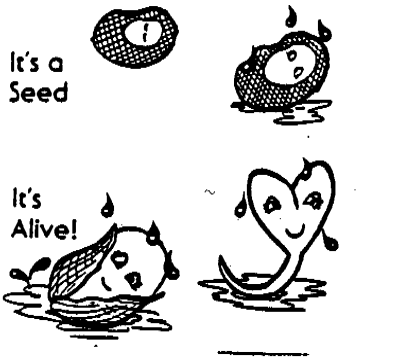
### Adzuki Bean Sprouts

The Adzuki bean comes from Japan, Korea and China. These little red beans produce sprouts that are delicious by themselves or in a mix with peppery lentil sprouts and pea sprouts.

### Other Beansprouts

Kidney, Pinto, and Navy beans are often sprouted to add variety to sprout mixes and recipes.

## GREEN-LEAF SPROUTS




The term "GREEN-LEAF SPROUTS" is often used to describe germinated vegetable and grain seeds. These sprouts are usually characterized by two tiny green leaves at the tip of a slender 1/2" to 3" shoot. Varieties of green leaf sprouts include:

**Alfalfa Sprouts**

Alfalfa Sprouts are probably the most widely available kind of sprout. Grown to an approximate height of 3 inches, Alfalfa Sprouts have two tender leaves on top of a pearly white shoot.

Alfalfa Sprouts are a nutritious alternative to lettuce in sandwiches and addition to any salad. These sprouts have a subtle flavor of fresh peas.

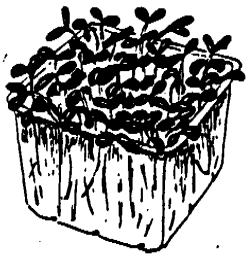
When purchasing Alfalfa Sprouts, look for fresh, crisp shoots of good color with perky green leaves. The greenness may vary in intensity. Pale green or slightly yellow tops are as tasty and tender as the darker leaves which have been exposed to more light, and contain more chlorophyll.

The bottom of the bag or cup should show white or pale tan roots. Brown tinge indicates the product is old and drying out. Mushy, darkened or bright yellow shoots should be discarded but won't affect the quality of the entire package.

When storing Alfalfa Sprouts, avoid temperature extremes (above 70 degrees and below 34 degrees). Alfalfa Sprouts do not freeze well. Sprouts can be revived by rinsing in cold water and shaking off the excess before returning to storage.

**Onion Sprouts**

A convenient way to get a pleasing taste of onion without the fuss is to use Onion Sprouts. Delicate little onion sprouts provide great companion tastes to hamburgers, tacos, and salads and are especially good with avocado.



**Radish Sprouts**

If you are a real radish fan, try a package of zippy Radish Sprouts. Quite "hot", this sprout offers the taste of radishes without the work.

**Clover Sprouts**

This sprout is similar to Alfalfa but each sprout is a sturdy baby clover plant. Try clover for a tasty change and use as you would Alfalfa Sprouts.

**Dill Sprouts**

Dill sprouts are grown with Alfalfa Sprouts to provide a delicate but interesting flavor to your salads or sandwiches. This product is especially good with tuna or egg salad.

**Garlic Sprouts**

Garlic was once thought to possess magical powers against evil and was widely used in charms and spells. This popular herb is often grown with Alfalfa Sprouts for a pleasing blend of flavors and crunch. Wonderful in turkey and meat sandwiches.

**Sunflower Sprouts**

Produced from Sunflower seeds, these sprouts have a mild, sweet flavor and crunchy consistency.

**Pea Sprouts**

Rising sugar content of pea sprouts causes the flavor to resemble the fresh pea taste much more than peas that are soaked and boiled. Wrinkled and smooth varieties of pea sprouts are equally nutritious and should be used when the sprout is about 1/2 inch long.

**Pumpkin Sprouts**

Pumpkin seeds are high in phosphorus, iron and protein. Hulled seeds are used to produce a wonderful tasting sprout that can be eaten raw or lightly toasted. Use them in salads, soups, bread and candy.

**Wheat Sprouts**

The most delicious of the grain sprouts, wheat sprouts should be used when they are about 1/2 inch long. Wheat sprouts cook quickly and can be used in recipes calling for whole grains.

**Lentil Sprouts**

Named "Lens" because their shape resembles the lens of a telescope, sprouted lentils have a peppery flavor. Used when 1/2 inch long, Lentil Sprouts are often used in soups, stews and casseroles.


## *ACKNOWLEDGEMENT*

***Dawn Cook**, who lives in an antique farmhouse in Rochester, Massachusetts, is the creative illustrator of our whole sprout family. We are real people who, for the most part, live in the area and work at **Jonathan's Sprouts**. She appears in this book only as a shadow passing lightly over the pages, but her humor, love for the earth, and gentle understanding of the broad range of human tastes and needs (especially in relation to sprouts) brings life to our humble offerings of recipes and suggestions. She poured her wit and wisdom into this work.*

***The International Sprout Growers Association** wishes to express its gratitude to **Dawn** for the many hours of painstaking work; drawing, typing and editing our recipe book. Several other people have contributed time and love, but in particular, we give thanks to the co-originator of the recipe book, **Judy Frasciana**, sales manager for **Jonathan's Sprouts**, who invented, collected, and edited so many of these recipes.*

170 Reprinted from Journal of Medicinal Chemistry, 1994, 37.


# Design and Synthesis of Bifunctional Isothiocyanate Analogs of Sulforaphane:† Correlation between Structure and Potency as Inducers of Anticarcinogenic Detoxication Enzymes‡

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*Received August 12, 1993*•

Thirty-five bifunctional isothiocyanates were synthesized as structural analogs of sulforaphane [(−)-1-isothiocyanato-4(*R*)-(methylsulfinyl)butane] that was recently isolated from broccoli as the principal and very potent inducer of detoxication (phase 2) enzymes in mouse tissues and murine hepatoma cells (Hepa 1c1c7) in culture (Zhang, Y.; Talalay, P.; Cho, C.-G.; Posner, G. H. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 2399–2403). Determination of the potency of each analog in inducing NAD(P)H:quinone reductase, a phase 2 detoxication enzyme, has allowed generalizations concerning the relation of structure and activity. The most potent analogs were bifunctional derivatives in which the isothiocyanate group was separated from a methylsulfonyl or an acetyl group by three or four carbon atoms, and in some of which these groups were conformationally restricted. Among these analogs, the bicyclic ketoisothiocyanate (±)-*exo*-2-acetyl-6-isothiocyanatonorbornane (**30**) was a very potent inducer (comparable to sulforaphane) of quinone reductase in hepatoma cells, and it also induced both quinone reductase and glutathione transferases in several mouse organs *in vivo*. This and related bicyclic ketoisothiocyanates represent potent phase 2 enzyme inducers that are relatively easily synthesized and that may be more stable metabolically than the natural sulfoxide sulforaphane.

The fate of chemical carcinogens *in vivo* is determined at least in part by the balance between phase 1 enzymes (cytochromes P-450) that activate many carcinogens to highly reactive electrophilic metabolites capable of damaging DNA and phase 2 enzymes (e.g. glutathione transferases, NAD(P)H:quinone oxidoreductase [QR], UDP-glucuronosyltransferases) that convert these reactive electrophiles to less toxic and more easily excretable products.[1-3] A wide variety of protectors against chemical carcinogenesis are also inducers of phase 2 enzymes in many animal cells and tissues, and there is convincing evidence that monofunctional induction of phase 2 enzymes is a major mechanism responsible for such protection. It is therefore of interest that vegetables, and especially crucifers, are rich in inducer activity and contain a variety of inducer molecules. By the use of a simple screening procedure involving measurement of quinone reductase activities of murine hepatoma cells grown in microtiter plates,[4,5] we have recently isolated and identified sulforaphane [(−)-1-isothiocyanato-4(*R*)-(methylsulfinyl)-butane] as the principal and very potent phase 2 enzyme inducer from SAGA broccoli.[6] A number of natural and synthetic isothiocyanates have been shown to block the neoplastic activity of a variety of carcinogens in rodents, to induce phase 2 enzymes *in vivo* and in cells in culture, and to inhibit metabolic activation of certain carcinogens.[7] In an effort to understand the unusually high potency of sulforaphane as an enzyme inducer, we designed, synthesized, and evaluated the activities *in vitro* of a number of isothiocyanates, each carrying one additional polar group. We also determined the inducer potency in mouse tissues of (±)-*exo*-2-acetyl-6-isothiocyanatonorbornane (**30**), one of the most potent synthetic analogs of sulforaphane developed in this study.

† These bifunctional isothiocyanates are the subject of a pending U.S. patent application.

‡ Abbreviations and trivial names: QR, quinone reductase [NAD(P)H:(quinone-acceptor) oxidoreductase, EC 1.6.99.2]; GST glutathione *S*-transferase, EC 2.5.1.18; CD value, the concentration of an inducer required to double the specific activity of quinone reductase in Hepa 1c1c7 murine hepatoma cells; CDNB, 1-chloro-2,4-dinitrobenzene; DCNB, 1,2-dichloro-4-nitrobenzene.

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• Abstract published in *Advance ACS Abstracts*, December 1, 1993.

## Results

**Acyclic Analogs of Sulforaphane in Which the Methylsulfinyl Group Has Been Replaced by Other Polar Groups.** We have recently shown that sulforaphane, isolated from broccoli, is an extremely potent inducer of QR, equivalent in potency to synthetic racemic sulforaphane.[6] The concentration of sulforaphane required to double the QR activity (CD value) was 0.2 μM. A limited structure–activity study of the analogs $CH_3S(O)_m(CH)_nN{=}C{=}S$, where $m = 0$, 1, or 2 and $n = 3$, 4, or 5, led to the following conclusions: (a) sulforaphane is the most potent inducer; (b) the sulfoxides and sulfones do not differ much in potency, but they are more potent than the sulfides; and (c) compounds with four or five methylene groups bridging the methylsulfur and isothiocyanate functions are more potent than those containing only three methylene groups.[6]

The polar sulfoxide group of sulforaphane (CD = 0.2 μM) is clearly very important for inducer activity, since *n*-hexyl isothiocyanate in which the sulfoxide functionality is replaced by a methylene group is much less potent (CD = 15 μM; Table 1). Measurements of the inducer potencies (Table 1) of analogs of the type $Z(CH_2)_4N{=}C{=}S$, synthesized for these studies, support the following conclusions: (a) of the eight synthetic analogs, only two are very potent; (b) of these two, the methyl ketone **4** and the dimethylphosphine oxide **8** are almost equal in potency to sulforaphane; and (c) whereas the methyl ketone analog

**Scheme 5**

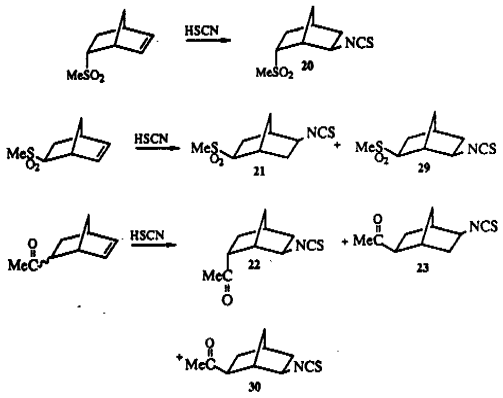
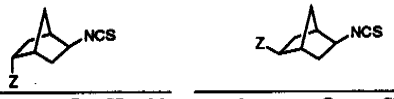



**Table 2.** Potency of Norbornyl Isothiocyanates in Inducing QR in Murine Hepatoma Cells

Four-Carbon Link between Functionalities



| compd | *endo* Z | CD (μM) | compd | *exo* Z | CD (μM) |
|---|---|---|---|---|---|
| | | | 21 | $MeSO_2$ | 0.7 |
| | | | 23 | MeCO | 0.4 |
| | | | 24 | N≡C | 0.6 |
| | | | 25 | $O_2N$ | 1.1 |
| | | | 26 | MeOOC | 0.7 |
| | | | 27 | MeCH(OH) | 0.5 |
| 28 | OH | 19 | | | |

Three-Carbon Link between Functionalities

| compd | *endo* Z | CD (μM) | compd | *exo* Z | CD (μM) |
|---|---|---|---|---|---|
| 20 | $MeSO_2$ | 1.0 | 29 | $MeSO_2$ | 0.2 |
| 22 | MeCO | 0.8 | 30 | MeCO | 0.3 |
| | | | 31 | MeOOC | 1.6 |

**Induction of Quinone Reductase and Glutathione Transferase in Mouse Tissues by Keto Isothiocyanate 30.** When synthetic (±)-*exo*-2-acetyl-6-isothiocyanatonorbornane (**30**) was administered to female CD-1 mice by gavage in doses of 7.5, 15, or 30 μmol daily for 5 days, the QR and GST (measured with both 1-chloro-2,4-dinitrobenzene and 1,2-dichloro-4-nitrobenzene), specific activities of the cytosols of liver, forestomach, glandular stomach, and proximal small intestine were increased in a dose-dependent manner (Figure 1). The increases in specific activities at the highest doses were generally about 2–4-fold, except for the inductions of GST in the small intestine (measured with DCNB) which were considerably higher (14–16-fold). We conclude that the keto isothiocyanate **30** induces QR not only in murine hepatoma cells, but also, like sulforaphane,[6] induces QR and GST activities in a number of murine organs. Insofar as quantitative comparisons can be made, keto isothiocyanate **30** and sulforaphane do not differ much in inducer potencies or organ-response patterns.

**Aromatic Analogs. A. Aryl Isothiocyanates.** As expected based on previous negative enzyme induction results with phenyl isothiocyanate,[3] none of the *ortho*-substituted phenyl isothiocyanates we have prepared (including *o*-OMe, -Cl, $-CH_2CH_2SMe$) has any significant inducer potency under standard assay conditions.

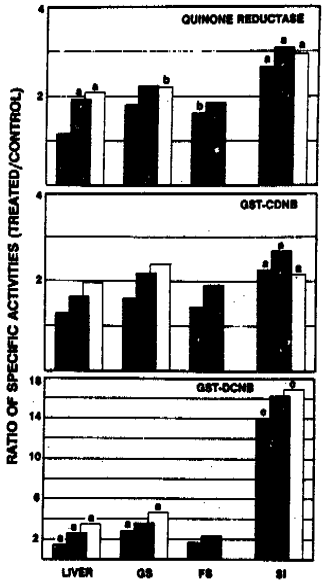


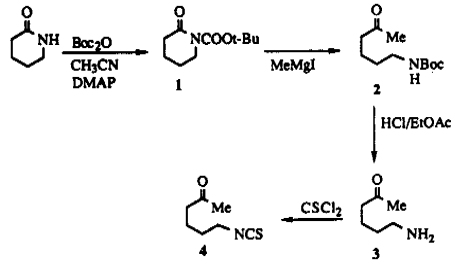

**Figure 1.** Effect of oral administration of *exo*-2-acetyl-6-isothiocyanatonorbornane (**30**) on the specific activities of cytosolic glutathione transferases measured with CDNB (GST-CDNB) and DCNB (GST-DCNB) and quinone reductase of liver, glandular stomach (GS), forestomach (FS), and proximal small intestine (SI) of mice. The compound was administered to 6-week-old female CD-1 mice in 0.1 mL of Emulphor EL620P (GAF, Linden, NJ) daily for 5 days in the following quantities: 7.5 μmol (solid bar), 15 μmol (hashed bar), or 30 μmol (open bar). Four or five animals were studied in each of the treated groups and 10 in the control group. The results are expressed as the ratios (± SEM) of the specific activities of organ cytosols from treated to controls receiving the vehicle only. Cytosols were prepared from the tissues 24 h after the last treatment and assayed for enzyme activities. The enzyme specific activities (nmol·$min^{-1}$·$mg^{-1}$ ± SEM) of the cytosols of control mice were as follows. Liver: GST-CDNB, 1080 ± 51.9; GST-DCNB, 10.9 ± 0.78; QR, 57.3 ± 4.1. Glandular stomach: GST-CDNB, 908 ± 27.5; GST-DCNB, 4.5 ± 0.18; QR, 2630 ± 190. Forestomach: GST-CDNB, 1780 ± 57.2; GST-DCNB, 7.6 ± 0.60; QR, 1040 ± 62.9. Small intestine: GST-CDNB, 685 ± 57.9; GST-DCNB, 0.89 ± 0.23; QR, 488 ± 45.9. The relative standard errors of the induction ratios [(SEM/mean) × 100] are as follows: no designation, ± 0–10%; *a*, ± 10–20%; *b*, ± 20–30%. The unknown reasons, the forestomachs of mice receiving 30 μmol of **30** were significantly heavier (89.4 ± 3.9 mg, wet weight) than the controls (42.4 ± 2.5 mg) or the forestomachs of mice receiving either 7.5 or 15 μmol of 30 (mean of 46.9 mg). For cytosols of the forestomach homogenates of animals receiving 30 μmol of **30** also contained more protein than the other groups. The specific activities of QR and GST in the cytosols of forestomachs of mice receiving the 30-μmol dose were considerably lower than those treated with the lower does of 30 and approached control values. The results obtained with the 30-μmol dose are omitted from the figure.

**B. Benzylic Isothiocyanates.** Prompted by a report by Kjaer on *o*-methoxybenzyl isothiocyanate,[9] we prepared a series of benzylic isothiocyanates substituted in the *ortho* position by the following substituents (CD values [μM] in parentheses): $CH_2SCH_3$ (4.3), OMe (2.4), $NMe_2$ (12.5), SMe (6.8), F (13.1), Cl (14.1), Br (12.5), Me (3.7), Et (12.5),

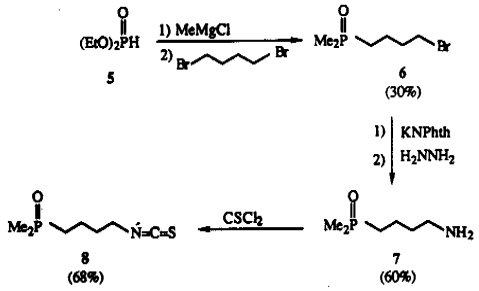
**Table 1. Effect of Replacing the Methylsulfinyl Group of Sulforaphane on Inducer Potency for QR in Murine Hepatoma Cells**

| Z | CD | Z | CD |
|---|---|---|---|
| Et | 15.0 | MeOOC | 2.8 |
| $CH_3S(O)$ (sulforaphane) | 0.2 | MeSCO | 2.8 |
| N≡C | 2.0 | MeCO | 0.2 |
| HOOC | 2.2 | *n*-BuCO | 2.0 |
| | | $Me_2P(=O)$ | 0.4 |
| $CH_3S(O)CH{=}CH(CH_2)_2NCS$ (sulforaphene) | 0.4 | | |

**Scheme 1**



**Scheme 2**



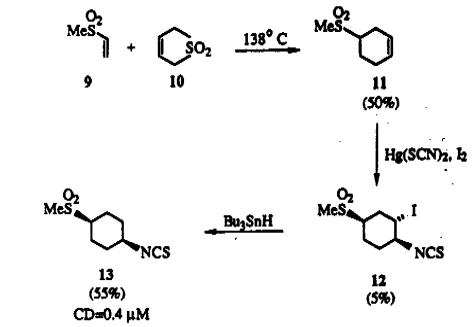
4 is a potent inducer, the corresponding *n*-butyl ketone is not a good inducer. At present, we do not understand the reason(s) for the differences in potencies among these analogs. The methods of preparation of the most potent analogs are outlined in Schemes 1 and 2.

**Nonaromatic Cyclic Analogs with Restricted Conformations. A. Monocyclic Analogs.** Cyclohexyl isothiocyanate is a relatively weak inducer (CD = 56 μM). Using general literature procedures, we prepared three methylsulfonyl isothiocyanates (13, 18, 19) in which the two polar functionalities are separated by four carbon atoms and in which a cyclohexane ring restricts the conformational mobility of the two functional groups (Schemes 3 and 4). Sulfones were targeted because they are more stable than sulfoxides toward several of the reaction conditions used in Schemes 3 and 4 and also because the sulfone erysolin, $CH_3SO_2(CH_2)_4NCS$, is only about 2-fold less potent than the sulfoxide sulforaphane.[6] The CD values of these conformationally restricted synthetic sulfonyl isothiocyanates 13, 18, and 19 ranged from 0.4 to 0.5 μM, indicating considerable potency and emphasizing the importance of the presence of the polar sulfonyl group, although not its spatial relation to the isothiocyanate function.

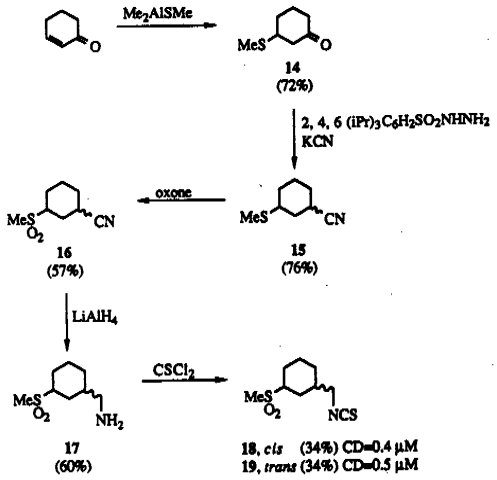
**B. Bicyclic Analogs.** Commercial *exo*-norbornyl isothiocyanate (CD = 32 μM) is a somewhat more potent



**Scheme 3**



**Scheme 4**



inducer than cyclohexyl isothiocyanate (CD = 56 μM). The dramatic increase in inducer potency observed by the addition of a methylsulfonyl group to the six-membered monocyclic system was also observed in the bicyclic norbornane analogs. Starting with commercially available 5-acetyl-2-norbornene or easily made 5-(methylsulfonyl)-2-norbornene, we added the elements of H-SCN across the strained carbon–carbon double bond of these norbornenes[8] to produce directly as the major products the mixture of positional and orientational isomers shown in Scheme 5. Chromatographic separations provided the pure bifunctional products listed in Table 2. Assignment of position and orientation of the two functional groups in each product was based on $^1H$ and $^{13}C$ NMR spectroscopy (see the Experimental Section); single-crystal X-ray crystallography confirmed the structure of bifunctional norbornane 23.

Several aspects of the data in Table 2 are noteworthy: (1) 9 of the 12 bifunctional compounds in this table have CD values of less than 1.0, indicative of high enzyme induction potency; (2) where direct comparison is possible, the compounds with the *exo*-oriented Z-substituents are more potent than those with *endo*-oriented Z-substituents (i.e. 29 > 20, 30 > 22); and (3) as was found in the acyclic series (*cf.* methyl ketone 4), the methyl ketone (i.e. acetyl) functionality in bicyclic analogs 22, 23, and 30 contributes significantly to making these bifunctional compounds very potent inducers.

OEt (2.5). None of these analogs was a particularly active inducer or even better than benzyl isothiocyanate itself (CD = 2–3 $\mu$M).

**Conclusion.** In conclusion, several easily prepared, conformationally restricted, non-sulfoxide, nonaromatic, bifunctional analogs of sulforaphane have been identified and have been shown *in vitro* to possess high phase 2 enzyme inducer potency. One of the most promising lead compounds, bicyclic keto isothiocyanate **30**, was shown also *in vivo* to induce QR and GST activities in a number of murine organs. Such keto isothiocyanates, probably more stable toward biological oxidation and reduction than sulfoxide–isothiocyanates like sulforaphane, may be useful for protection against cancer.

## Experimental Section

**General Methods.** Thiophosgene was purchased from Carbolabs Inc. (Bethany, CT), and all other reagents were purchased from Aldrich Chemical Co. (Milwaukee, WI) and, unless otherwise specified, were used as received without further purification. Benzyl isothiocyanate, obtained from the Aldrich Chemical Co., was distilled under reduced pressure prior to use. Other isothiocyanates which were not synthesized for this project were obtained from Trans World Chemicals (Rockville, MD). $^1$H and $^{13}$C NMR spectra were determined on a Bruker AMX 300 MHz or a Varian XL-400 MHz spectrometer. High-resolution mass spectra were obtained at 70 eV on a VG-70S mass spectrometer. FT-IR spectra were recorded on a Perkin-Elmer Model 1600 FT-IR spectrophotometer. Elemental analyses were performed by Atlantic Microlab, Norcross, GA. Medium-pressure column chromatography was performed with silica gel (EM SCIENCE, 230–400 mesh). High-pressure liquid chromatography was performed with RAININ HPXL chromatography using a semi-prep silica column.

**Boc-Protected Lactam 1.** To a flask charged with 0.56 g (5.7 mmol) of $\delta$-valerolactam, 60 mg (0.5 mmol) of 4-(dimethylamino)-pyridine (DMAP), and 30 mL of acetonitrile was added 1.22 g (5.7 mol) of di-*tert*-butyl dicarbonate ($Boc_2O$) at room temperature (RT). After 4 h at RT, the reaction mixture was concentrated and partitioned between ether and 1 M $KHSO_4$. The separated organic layer was washed with saturated $NaHCO_3$ and brine, dried over $MgSO_4$, and concentrated to give 0.90 g of product as a bright yellowish green liquid (used for the next reaction without purification).

**4-Boc-aminobutyl Methyl Ketone (2).** To protected lactam 1 in 10 mL of THF was added 2 mL of MeMgI (3 M in ether, 6 mmol) slowly at −78 °C. After 3 h at −78 °C, the reaction mixture was quenched with aqueous $NH_4Cl$, and the resulting solution was extracted with ether (2 × 20 mL). The combined ether solution was washed with brine, dried over $MgSO_4$, and concentrated in vacuo to give crude methyl ketone 2 as a brown oil (used for the next reaction without further purification).

**4-Aminobutyl Methyl Ketone (3).** To methyl ketone 2 dissolved in 3 mL of EtOAc was added 1 mL of 37% of HCl at RT. After 30 min, the reaction mixture was diluted with 5 mL of $H_2O$ and washed with ether. The aqueous solution was then strongly basified with solid NaOH and extracted with $CHCl_3$ (2 × 20 mL). The organic solution was dried over $K_2CO_3$ and concentrated to give a smelly light brown oil (used for the next reaction without purification).

**2-Oxohexyl Isothiocyanate (4).** To amino methyl ketone 3 in 2 mL of $H_2O$ and 2 mL of $CHCl_3$ were added 0.16 mL of $CSCl_2$ (1.58 mmol) and 2 mL of 5% NaOH at RT. After 30 min the reaction mixture was diluted with 10 mL of $CHCl_3$, and the decanted organic layer was dried over $MgSO_4$, concentrated, and chromatographed (8/2 Hex/EtOAc) to give 42 mg of isothiocyanate 4 as a brown oil in an overall yield of 6% from lactam 1: $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 3.54–3.49 (m, 2H), 2.53–2.46 (m, 2H), 2.16 (s, 3H), 1.72–1.68 (m, 4H); FT-IR ($CHCl_3$) 3019, 2191, 2112, 1715, 1224 cm$^{-1}$; $^{13}$C NMR (400 MHz, CD $Cl_3$) $\delta$ 207.8, 44.8, 42.4, 29.9, 29.3, 20.6; HRMS calcd for $C_7H_{11}NOS$ 157.0561, found 157.0565.

**(4-Bromobutyl)dimethylphosphine Oxide (6).** To a 25-mL flame-dried round-bottomed flask charged with 15.2 mL (45.6 mmol) of MeMgCl (3.0 M in THF) was added 1.5 mL (11.41 mmol) of diethyl phosphite (5) while the internal temperature was maintained around 25 °C with occasional cooling with an ice–water bath. After 1 h, the mixture was cannulated into a separate flask charged with 2.55 mL (22.82 mmol) of 1,4-dibromobutane and 15 mL of THF at 0 °C under an Ar atmosphere. Upon addition, the reaction mixture was heated under reflux for 5 h, cooled, and dumped into 30 mL of cold dilute HCl. The resulting aqueous solution was extracted with $CHCl_3$ (3 × 50 mL), and the combined organic solution was washed with saturated $K_2CO_3$, dried over $K_2CO_3$, and concentrated in vacuo to give 2.48 g of crude product as a tan oil. Purification by flash column chromatography (silica gel, 8/2 EtOAc/MeOH → 6/4 EtOAc/hexane) afforded 0.72 g of phosphine oxide 6 as a colorless oil (used for the next reaction without purification).

**(4-Aminobutyl)dimethylphosphine Oxide (7).** In a 100-mL round-bottomed flask were placed 0.733 g (3.44 mmol) of phosphine oxide 6, 0.766 g of potassium phthalimide, and 20 mL of DMF. The mixture was heated under reflux for 4 h, cooled, and dumped into 60 mL of $CHCl_3$. The organic solution was washed with $H_2O$, dried over $NaHCO_3$, and concentrated in vacuo to afford 0.92 g of product phthalimide as a white solid. To a separate flask charged with 0.10 g of product phthalimide was added 4 mL of methanolic hydrazine (0.2 M in MeOH) at RT. After 14 h at RT, the reaction mixture was concentrated, and the residue was treated with 5 mL of 1 N HCl, washed with $CHCl_3$, and strongly basified with solid NaOH. The basified solution was then extracted with $CHCl_3$ (2 × 20 mL), and the combined organic solution was dried over $K_2CO_3$ and concentrated in vacuo to give 33 mg of amine phosphine oxide 7 as a white solid (used for the next reaction without further purification).

**(4-Isothiocyanatobutyl)dimethylphosphine Oxide (8).** To a flask charged with 33 mg (0.22 mmol) of amine 7 and 1 mL of $CHCl_3$ were added at RT 0.02 mL (0.27 mmol) of $CSCl_2$ and 0.3 mL of 1 N NaOH. After 35 min at RT, the reaction mixture was partitioned between 10 mL of $CHCl_3$ and 10 mL of $H_2O$. The separated organic layer was dried over $MgSO_4$, concentrated in vacuo, and chromatographed (8/2 EtOAc/MeOH) to afford 29 mg of isothiocyanate 8 as a reddish yellow oil in 68% yield: $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 3.54 (t, $J$ = 6.0 Hz, 2H), 1.82–1.70 (m, 6H), 1.48 (s, 3H), 1.44 (s, 3H); FT-IR ($CHCl_3$) 2941, 2191, 2097, 1302, 1173 cm$^{-1}$; $^{13}$C NMR (400 MHz, $CDCl_3$) $\delta$ 44.5, 30.6 (d, $J$ = 20.2 Hz, 1C), 30.7 (d, $J$ = 34.7 Hz, 1C), 19.3, 16.2 (d, $J$ = 69 Hz, 2C); $^{31}$P NMR ($CDCl_3$) $\delta$ 46.1; HRMS calcd for $C_7H_{14}NOPS$ 191.0534, found 195.0536.

**4-(Methylsulfonyl)cyclohexene (11).** In a sealed tube were placed 0.50 g (5.5 mmol) of methyl vinyl sulfone (9), 0.67 g (5.5 mmol) of 1,4-but-2-enediyl sulfone (10) and 2 mL of absolue EtOH. After 2 days at 138 °C, the reaction mixture was cooled and poured into aqueous $Na_2CO_3$. After 10 min with vigorous stirring, the aqueous solution was extracted with ether (2 × 10 mL), dried over $MgSO_4$, concentrated in vacuo, and chromatographed (1/1 hexane/ether) to give 0.20 g (2.2 mmol, 40% recovery) of methyl vinyl sulfone (9) and 0.40 g of sulfone 11 as a brown oil in 50% yield.

**Iodo Isothiocyanate 12.** To a flask charged with 342 mg (0.63 mmol) of $Hg(SCN)_2$ was added a premixed solution of $I_2$ in 8 mL of benzene. After 30 min at RT, to this mixture was added 202 mg (1.26 mmol) of cyclohexene 11 dissolved in 1 mL of benzene, and the flask containing the reaction mixture was wrapped with aluminum foil and stirred for 7.5 days at RT under an argon atmosphere. The reaction mixture was then filtered off, and the solid material was washed with ether. The ether solution was washed with aqueous KI, aqueous $Na_2S_2O_3$, and brine successively, dried over $MgSO_4$, and concentrated in vacuo. Flash column chromatography (1/1 ether/hexane) afforded 20 mg of iodide 12 (5% yield) as a brown oil along with three other isomers (11% yield).

**Sulfonyl Isothiocyanate 13.** To a flask charged with 21 mg (0.07 mmol) of iodide 12 and 1 mL of benzene was added 0.05 mL (0.2 mmol, 3 equiv) of $Bu_3SnH$ at RT. After 10 h, the reaction mixture was treated with 2 mL of wet ether and 35 mg (0.2 mmol) of DBU (1,8-diazabicyclo[5.4.0]undec-7-ene). The resulting mixture was filtered off, concentrated in vacuo and chromato-

graphed (100% ether → 1/1 ether/EtOAc) to afford 7.3 mg of isothiocyanate **13** as white solid (mp 123 °C) in 55% yield: $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 4.11–4.08 (m, 1H), 2.85 (s, 3H), 2.87–2.80 (m, 1H, overlapped), 2.23–2.18 (m, 4H), 1.93 (dd, $J$ = 14.8, 4.4 Hz, 1H), 1.87 (dd, $J$ = 13.2, 3.2 Hz, 1H), 1.67 (tt, $J$ = 13.2, 3.6 Hz, 2H); FT-IR ($CHCl_3$) 3025, 2943, 2261 , 2085, 1302 cm$^{-1}$; $^{13}$C NMR ($CDCl_3$) $\delta$ 133.5, 61.2, 52.3, 36.8, 30.5, 20.6; HRMS calcd for $C_8H_{13}NO_2S_2$ 219.0388, found 219.0391.

**3-Cyanocyclohexyl Methyl Sulfide (15).** Into a 100-mL round-bottomed flask were placed 0.438 g (3.0 mmol) of 14,[11] 1.418 g (4.8 mmol) of 2,4,6-triisopropylbenzenesulfonyl hydrazide,[10] and 8 mL of MeOH at RT. After 1 h, 0.739 g (11.3 mmol) of KCN was added at RT, and the resulting mixture was heated under gentle reflux for 3 h. The reaction mixture was cooled, diluted with 20 mL of $H_2O$, and extracted with $CH_2Cl_2$ (2 × 20 mL). The organic solution was washed with aqueous $NaHCO_3$, dried over $MgSO_4$, concentrated in vacuo, and purified by flash column chromatography (8/2 hexane/EtOAc) to afford 0.360 g of sulfide **15** as a yellow oil in 76% yield.

**3-Cyanocyclohexyl Methyl Sulfone (16).** To a flask charged with 0.36 g (2.32 mmol) of sulfide **15** and 10 mL of aqueous MeOH (9/1 v/v MeOH/$H_2O$) was added 2.75 g (4.64 mmol) of Oxone (Aldrich, $2KHSO_5{\cdot}KHSO_4{\cdot}K_2SO_4$) at RT. After 24 h, the reaction mixture was filtered through a sintered-glass funnel, and the filtered solid material was washed with 50 mL of $CHCl_3$. The combined organic solution was washed with $H_2O$, dried over $MgSO_4$, and concentrated in vacuo to afford 0.246 g of sulfone **16** (57% yield) as a colorless oil. This material was used in the next reaction without purification.

**3-(Aminomethyl)cyclohexyl Methyl Sulfone (17).** To a suspension of 0.098 g (2.59 mmol) of $LiAlH_4$ in 10 mL of anhydrous ether was cannulated 0.246 g (1.31 mmol) of nitrile **16** dissolved in 3 mL of THF at RT. Upon addition, the reaction mixture was heated under reflux. After 2.5 h, the reaction mixture was cooled, quenched with 0.5 mL of $H_2O$ and 0.5 mL of 5% NaOH, and filtered through a sintered-glass funnel. The solid material filtered was thoroughly washed with ether. The combined organic solution was dried over $K_2CO_3$ and concentrated in vacuo to afford 0.150 g of amine **17** (60% yield) as a colorless oil. This material was used in the next reaction without purification.

***cis*-(3-(Methylsulfonyl)cyclohexyl)methyl Isothiocyanate 18 and *trans*-(3-(Methylsulfonyl)cyclohexyl)methyl Isothiocyanate 19.** To a flask charged with 0.15 g (0.78 mmol) of amine **17** and 3 mL of $CHCl_3$ were added 0.07 mL (0.92 mmol) of $CSCl_2$ and 1.5 mL of 5% NaOH at RT. After 1 h, the reaction mixture was diluted with 10 mL of $CH_2Cl_2$, washed with $H_2O$ and brine, dried over $MgSO_4$, concentrated in vacuo, and chromatographed (1/1 hexane/EtOAc) to give 0.123 g of products (67% yield) as a mixture of isothiocyanates **18** and **19** (1:1 ratio). HPLC (40/60 EtOAc/hexane) separation afforded analytically pure **18** and **19** (both as a colorless oil). **18**: $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 3.47 (d, $J$ = 6.0 Hz, 2H), 2.92–2.82 (m, 1H), 2.84 (s, 3H), 2.28–2.20 (m, 2H), 2.04 (tt, $J$ = 6.8, 3.0 Hz, 1H), 1.87–1.75 (m, 2H), 1.53–1.27 (m, 3H), 1.06 (tq, $J$ = 12.2, 3.6 Hz, 1H); FT-IR ($CHCl_3$) 3025, 2931, 2861, 2191, 2097, 1449, 1308 cm$^{-1}$; $^{13}$C NMR ($CDCl_3$) $\delta$ 56.5, 45.5, 32.6, 32.5, 23.9, 23.8, 20.0, 19.1; HRMS calcd for $C_9H_{15}NO_2S_2$ 233.0544, found 233.0548. **19**: $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 3.50 (d, $J$ = 6.8 Hz, 2H), 3.09–3.03 (m, 1H), 2.88 (s, 3H), 2.45–2.37 (m, 1H), 2.14–2.07 (m, 1H), 1.98–1.84 (m, 4H), 1.74–1.66 (m, 1H), 1.59–1.41 (m, 2H); FT-IR ($CHCl_3$) 3013, 2943, 2872, 2191, 2097, 1449, 1308 cm$^{-1}$; $^{13}$C NMR ($CDCl_3$) $\delta$ 52.6, 43.5, 33.6, 28.0, 22.5, 22.1, 19.5, 14.9; HRMS calcd for $C_9H_{15}NO_2S_2$ 233.0544, found 233.0545.

**2-*endo*-Methylsulfonyl Isothiocyanate 20.** In a 20-mL hydrolysis tube were placed 349 mg (2.03 mmol) of 5-*endo*-(methylsulfonyl)-2-norbornene,[12] 394 mg (4.05 mmol) of KSCN, 319 mg (3.25 mmol) of $H_2SO_4$, and 0.1 mL of $H_2O$ at RT via literature precedent.[13] The tube was sealed and shaken vigorously for 5 min to give a finely divided yellow suspension. After 4 days at 50 °C, the reaction mixture was filtered through a sintered-glass funnel, and the brown solid was washed with ether. The combined organic solution was washed with $H_2O$, brine, dried over $MgSO_4$, and concentrated to give 270 mg of crude product containing unreacted starting material as the major component. Flash column chromatography (1/1 hexane/EtOAc) afforded 47 mg of isothiocyanate **20** as a white solid. Recrystallization from a 2:1 mixture of hexane and ether gave 23 mg of isothiocyanate **20** (5% yield) as white needles (mp 110–111 °C). The *endo* orientation of the methylsulfonyl group was assigned based on its $^1$H NMR chemical shift ($\delta$ 2.84) being upfield from that of the corresponding *exo* methylsulfonyl groups of **21** and **28** ($\delta$ 2.86–2.87).[14] The 1,3-relationship of the two substituents was assigned based on the difference in chemical shifts between the bridgehead hydrogens ($\Delta\delta$ = 0.2) vs that in the 1,4-positional isomer **21** ($\Delta\delta$ = 0.1):[15] $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 3.79 (dd, $J$ = 7.6, 4.4 Hz, 1H), 3.30–3.25 (m, 1H), 2.90–2.84 (m, 1H), 2.84 (s, 3H), 2.68 (d, $J$ = 4.4 Hz, 1H), 2.02 (ddd, $J$ = 13.1, 10.1, 4.2 Hz, 1H), 1.90–1.86 (m, 1H), 1.72–1.52 (m, 4H); FT-IR ($CHCl_3$) 3 025, 2120, 2097, 1320 cm$^{-1}$. Anal. ($C_9H_{13}NO_2S_2$) C, H, N, S.

**2-*exo*-Methylsulfonyl Isothiocyanate 21 and 2-*exo*-Methylsulfonyl Isothiocyanate 29.** The same procedure as described for **20** was used except that the reaction mixture was stirred for 6 days at 65 °C. After workup, isothiocyanates **21** (17% yield) and **29** (5% yield) were isolated by flash column chromatography (100% ether → 100% EtOAc). Isothiocyanate **21** was recrystallized from $CH_2Cl_2$/ether/hexane to afford ivy leaf-shaped crystals (mp 142–143 °C) in 12% yield. Isothiocyanate **29** was recrystallized from ether to afford small needles (mp 82–82.5 °C) in 4% yield. The assignment of positional isomers was done as described for isothiocyanate **20**. Isothiocyanate **21**: $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 3.66 (t, $J$ = 6.8 Hz, 1H), 2.90 (bs, 1H), 2.86 (s, 3H), 2.80 (dd, $J$ = 8.0, 5.2 Hz, 1H), 2.66 (bd, $J$ = 5.2 Hz, 1H), 2.12 (td, $J$ = 14.0, 5.2 Hz, 1H), 2.03 (dt, $J$ = 12.0, 2.2 Hz, 1H), 1.88–1.84 (m, 2H), 1.68–1.60 (m, 2H); FT-IR ($CHCl_3$) 3025, 2120, 2073, 1320 cm$^{-1}$. Anal. ($C_9H_{13}NO_2S_2$) C, H, N, S. Isothiocyanate **29**. $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 3.65 (dd, $J$ = 6.8, 2.8 Hz, 1H), 2.98 (bs, 1H), 2.87 (s, 3H), 2.76 (dd, $J$ = 6.8, 1.2 Hz, 1H), 2.58 (bs, 1H), 2.06–1.61 (m, 6H); FT-IR ($CHCl_3$) 3025, 2978, 2191, 2120, 2085, 1349, 1308, 1138 cm$^{-1}$; $^{13}$C NMR (400 MHz, $CDCl_3$) $\delta$ 132.2, 61.5, 58.0, 45.5, 39.6, 39.2, 35.3, 33.7, 31.2; HRMS calcd for $C_9H_{13}NO_2S_2$ 231.0388, found 231.0390.

**2-*endo*-Acetyl Isothiocyanate 22, 2-*exo*-Acetyl Isothiocyanate 23, and 2-*exo*-Acetyl Isothiocyanate 30.** To a 100-mL three-neck round-bottomed flask equipped with a magnetic stirring bar, dropping funnel, and reflux condenser were placed 2.0 g (14.7 mmol) of 5-acetyl-2-norbornene (mixture of *endo* and *exo*, Aldrich Chemical Co.), 2.86 g (29.4 mmol) of KSCN, and 10 mL of benzene. To this solution was added at RT a mixture of 2.1 g (21.5 mmol) of concentrated sulfuric acid and 1.0 mL of water slowly using a dropping funnel. After 4 days at 50 °C, the reaction mixture was filtered through a sintered-glass funnel. The filtered white solid was washed with 50 mL of ether. The combined organic solution was then washed with water and brine successively, dried over $MgSO_4$, and concentrated in vacuo to afford a tan oil. Subsequent purification via flash column chromatography (2/8 ether/hexane) afforded 1.73 g of product (60% yield, colorless oil) as a mixture of four stereoisomers. Purification by HPLC (97/3 hexane/EtOAc, 10 mL/min) gave isothiocyanates **22** (10% yield), **23** (23% yield), and **30** (22% yield). The assignment of positional and orientational isomers was done as described for isothiocyanates **20**, **21**, and **29**. The structure of isothiocyanate **23** was confirmed by X-ray crystallography. Isothiocyanate **22**: $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 3.51 (dd, $J$ = 7.6, 2.8 Hz, 1H), 2.85–2.79 (m, 1H), 2.68–2.54 (m, 1H), 2.43 (d, $J$ = 4.8 Hz, 1H), 2.06 (s, 3 H), 1.74–1.70 (m, 1H), 1.66 (ddd, $J$ = 13.6, 7.6, 2.4 Hz, 1H), 1.59–1.43 (m, 4H); FT-IR ($CHCl_3$) 3013, 2955, 2132, 2097, 1702, 1343 cm$^{-1}$. Anal. ($C_{10}H_{13}NOS$) C, H, N, S. Isothiocyanate **23**: $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 3.60 (dd, $J$ = 7.2, 2.8 Hz, 1H), 2.57 (d, $J$ = 4.4 Hz, 1H), 2.51 (d, $J$ = 4.8 Hz, 1H), 2.34 (dd, $J$ = 8.8, $J$ = 5.2 Hz, 1H), 2.15 (s, 3H), 1.98 (dt, $J$ = 13.2, 4.8 Hz, 1H), 1.88 (ddd, $J$ = 13.2, 7.6, 2.4 Hz, 1H), 1.75 (dt, $J$ = 13.6, 4.4 Hz, 1H), 1.52 (ddt, $J$ = 10.8, 4.0, 1.6 Hz, 1H), 1.33 (ddt, $J$ = 10.8, 4.0, 1.6 Hz, 1H), 1.22 (ddd, $J$ = 13.2, 8.8, 2.0 Hz, 1H); FT-IR ($CHCl_3$) 2978, 2 179, 2146, 2085, 1708, 1449, 1343 cm$^{-1}$. Anal. ($C_{10}H_{13}NOS$) C, H, N, S. Isothiocyanate **30**: $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 3.64 (dd, $J$ = 7.6, 2.8 Hz, 1H), 2.71 (bs, 1H), 2.43 (dd, $J$ = 4.5, 3.6 Hz, 1H), 2.31 (dd, $J$ = 8.4, 6.0 Hz, 1H), 2.17 (s, 3H), 1.83–1.67 (m, 2H), 1.58–1.54 (m, 2H), 1.38–1.30 (m, 2H); $^{13}$C NMR ($CDCl_3$) $\delta$ 207.4, 130.1, 58.3, 50.7, 46.5, 39.9, 35.4, 33.6, 31.5, 28.7; FT-IR ($CHCl_3$) 2955, 2132, 2085, 1708, 1343 cm$^{-1}$; HRMS calcd for $C_{10}H_{13}NOS$ 195.0719, found 195.0719.

**2-*exo*-Cyanonorbornyl Isothiocyanate 24.** To a sealed tube charged with 145 mg (1.2 mmol) of 5-*exo*-cyano-2-norbornene, 238 mg (2.4 mmol) of KSCN, and 3 mL of benzene were added 177 mg (2.4 mmol) of $H_2SO_4$ and 0.08 mL of $H_2O$. After 48 h at 63 °C, the reaction mixture was diluted with ether, filtered, concentrated, and chromatographed (3/7 ether/hexane) to give 77 mg (53%) of starting material and 33 mg of product as a mixture of two isomers. Subsequent HPLC separation (9/1 hexane/EtOAc) afforded 30 mg of isothiocyanate 24 as a white solid (mp 49.5–50.5 °C from hexane) in 14% yield: $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 3.59 (t, $J$ = 5.6 Hz, 1H), 2.73 (bs, 1H), 2.64 (d, $J$ = 4.4 Hz, 1H), 2.32 (ddd, $J$ = 9.2, 4.8, 1.6 Hz, 1H), 1.90 (dt, $J$ = 13.6 Hz, 4.8, 1H), 1.85–1.73 (m, 5H); FT-IR ($CHCl_3$) 3021, 2979, 2954, 2240, 2201, 2146, 2100, 1452, 1349 cm$^{-1}$; $^{13}$C NMR (400 MHz, $CDCl_3$) $\delta$ 131.0, 122.2, 57.3, 43.4, 40.8, 39.0, 34.4, 31.8, 29.9; HRMS calcd for $C_9H_{10}N_2S$ 178.0563, found 178.0561.

**2-*exo*-Nitronorbornyl Isothiocyanate 25.** To a flask charged with 183 mg (1.3 mmol) of 5-*exo*-nitro-2-norbornene,[16] 255 mg (2.6 mmol) of KSCN, and 6 mL of benzene were added 190 mg (2.6 mmol) of $H_2SO_4$ and 0.1 mL of $H_2O$. After 75 h at 40 °C, the reaction mixture was diluted with ether, filtered off, concentrated, and chromatographed (9/1 hexane/ether) to afford 107 mg of starting material (59%) and 36 mg of isothiocyanate 25 as a yellow solid (14%). Subsequent recrystallization from ether/hexane gave 26 mg of isothiocyanate 25 as colorless needles (mp 67–68 °C) in 10% yield: $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 4.32 (q, $J$ = 8.0 Hz, 1H), 3.62 (t, $J$ = 5.2 Hz, 1H), 3.00 (bs, 1H), 2.67 (d, $J$ = 4.8 Hz, 1H), 2.39 (dt, $J$ = 14.4, 4.0 Hz, 1H), 1.88–1.71 (m, 5H); FT-IR ($CHCl_3$) 3013, 2132, 2085, 1549, 1367, 1220 cm$^{-1}$. Anal. ($C_8H_{10}N_2O_2S$), C, H, N, S.

**2-*exo*-(Methoxycarbonyl)norbornyl Isothiocyanate 26 and 2-*exo*-(Methoxycarbonyl)norbornyl Isothiocyanate 31.** To a flask charged with 40 mg (0.26 mmol) of 5-*exo*-(methoxycarbonyl)-2-norbornene,[17] 51 mg (0.53 mmol) of KSCN, and 0.5 mL of benzene were added 38 mg (0.53 mmol) of $H_2SO_4$ and 0.02 mL of $H_2O$. After 46 h at 63 °C, the reaction mixture was directly chromatographed (7/3 hexane/ether) to give 20 mg (0.09 mmol) of product as a mixture of two isomers based on $^1$H NMR analysis (26:31 85:15). Purification by HPLC (95/5 hexane/EtOAc) afforded pure isothiocyanates 26 as a white solid (ca. 31% yield, mp 39.5–40.5 °C) and 31 as a liquid (ca. 6% yield). Isothiocyanate 26: $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 3.67 (s, 3H), 3.59 (dd, $J$ = 7.6, 3.2 Hz, 1H), 2.63 (d, $J$ = 3.5 Hz, 1H), 2.55 (d, $J$ = 4.3 Hz, 1H), 2.25 (dd, $J$ = 8.5, 4.3 Hz, 1H), 1.97 (dt, $J$ = 13.7, 4.7 Hz, 1H), 1.85 (ddd, $J$ = 13.4, 7.8, 1.9 Hz, 1H), 1.74 (dt, $J$ = 13.5, 3.8 Hz, 1H), 1.62–1.53 (m, 2H), 1.42–1.36 (m, 1H); FT-IR ($CHCl_3$) 2976, 2953, 2140, 2088, 1729, 1437, 1346 cm$^{-1}$; $^{13}$C NMR (400 MHz, $CDCl_3$) $\delta$ 175.2, 58.0, 51 .9, 45.0, 43.8, 40.2, 40.1, 33.6, 29.8; HRMS calcd for $C_{10}H_{13}NO_2S$ 211.0667, found 211.0669. 31: $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 3.68 (s, 3H), 3.62 (dd, $J$ = 7.6, 3.6 Hz, 1H), 2.76 (bs, 1H), 2.44 (bs, 1H), 2.22 (dd, $J$ = 8.4, 5.6 Hz, 1H), 1.88–1.40 (m, 6H); FT-IR ($CHCl_3$) 3027, 3009, 2974, 2954, 2197, 2132, 1732, 1437, 1346 cm$^{-1}$; $^{13}$C NMR (400 MHz, $CDCl_3$) $\delta$ 174.7, 58.2, 52.1, 47.8, 42.8, 39.8, 35.4, 34.0, 33.0; HRMS calcd for $C_{10}H_{13}NO_2S$ 211.0667 found 211.0667.

***exo*-2-(1′-Hydroxyethyl)norbornyl Isothiocyanate 27.** To 37.3 mg (0.2 mmol) of acetyl isothiocyanate 23 in 1.5 mL of MeOH was added 8.7 mg (0.2 mmol) of $NaBH_4$ slowly at 0 °C. After 15 min at 0 °C, the reaction mixture was treated with a few drops of water, diluted with ether, dried over $MgSO_4$, concentrated in vacuo, and purified by prep TLC (8/2 ether/hexane) to give 21.0 mg of hydroxy isothiocyanate 27 as a mixture of diastereomers (white solid; mp 64–69 °C recrystallized from hexane) in 56% yield: $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.19 (d, $J$ = 6.0 Hz, $CH_3$), 1.10 (d, $J$ = 6.4 Hz, $CH_3$); FT-IR ($CHCl_3$) 3623 , 3460, 2966, 2872, 2097, 1343 cm$^{-1}$. Anal. ($C_{10}H_{15}NOS$), C, H, N, S.

**2-*endo*-Hydroxynorbornyl Isothiocyanate 28.** To a flask charged with 0.515 g (4.54 mmol) of 5-hydroxy-2-norbornene (mixture of *endo* and *exo*, Aldrich Chemical Co.) 0.530 g (5.45 mmol) of KSCN, and 8 mL of benzene was added a premixed solution of $H_2SO_4$ (0.400 g) in 0.2 mL of $H_2O$ at RT. After 3 days at 55 °C, the reaction mixture was filtered and chromatographed (40/60 ether/hexane) to give 0.075 g of product as a white solid in 10% yield. A portion of the material was recrystallized from hexane/ether for analysis (white flakes; mp 64–66 °C): $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 4.24–4.18 (m, 1H), 3.67–3.65 (m, 1H), 2.52 (ddd, $J$ = 13.2, 7.6, 2.0 Hz, 1H), 2.41 (d, $J$ = 5.2 Hz, 1H), 2.35 (td, $J$ = 5.2, 1.6 Hz, 1H), 2.00 (ddd, $J$ = 15.6, 10.4, 5.6 Hz, 1H), 1.69 (dq, $J$ = 14.0, 3.2 Hz, 1H), 1.57–1.53 (m, 2H), 1.45–1.42 (m, 1H), 0.76 (dt, $J$ = 14.0, 3.2 Hz, 1H); FT-IR ($CHCl_3$) 3613 , 3472, 2966, 2097, 1343 cm$^{-1}$. Anal. ($C_9H_{11}NOS$) C, H, N, S.

**Bioassay Procedures. Measurement of Inducer Potency in Hepta 1c1c7 Murine Hepatoma Cells.** These determinations were carried out on cells grown in 96-well microtiter plates according to minor modifications[18] of the procedure of Prochask© and Santamaria.[4] The cells (10 000 per well) were grown for 24 h in medium containing 10% heat- and charcoal-treated fetal calf serum and then exposed to serial dilutions of the inducers for 48 h before measurement of QR specific activity. Compounds were dissolved in acetonitrile and diluted so that the final concentration of solvent was 0.1% by volume in all wells.

**Induction of Quinone Reductase and Glutathione Transferase Activities in Mouse Tissues. Treatment of Animals.** Five-week-old female CD-1 mice (Charles River Laboratories, Wilmington, MA) were acclimatized for 1 week on AIN 76A pellets diet. The animals were housed in plastic cages (four or five per cage). Each mouse received 0.1 mL of Emulphor EL620P (GAF, Linden, NJ) alone (10 mice) or 0.1 mL of Emulphor containing 7.5 $\mu$mol (4 mice), 15 $\mu$mol (5 mice), or 30 $\mu$mol (5 mice) of *exo*-2-acetyl-6-isothiocyanatonorbornane, daily by gavage for 5 days. Twenty-four hours after the last treatment, the animals were killed by carbon dioxide inhalation, the organs were removed, frozen in liquid nitrogen, and stored at −80 °C until analysis.

**Preparation of Tissue Cytosols and Assay of Their Enzymatic Activities.** The cytosols were prepared as described.[18,19] In the present experiments the entire proximal small intestine (after removal of contents) was homogenized rather than the mucosal scrapings.

Specific enzyme activities were measured at 25 °C as described[18,20] except that the assay systems were miniaturized (to one-tenth volume) so that measurements could be made in 96-well microtiter plates with the use of a microtiter plate reader (UVmax, Molecular Devices, Palo Alto, CA). The QR specific activities were determined in a final volume of 0.3 mL by measuring the NADPH-dependent rate of menadiol-mediated reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) at 610 nm. The specific activities of GST were measured with DCNB and CDNB in final volumes of 0.2 mL at 340 nm. Suitable rates were obtained by use of appropriate volumes and dilutions of cytosols and were derived from absorbance changes during the initial 2 min, based on the average of four wells. Rates were corrected for absorbance changes in wells containing all components except cytosol. Protein determinations were made according to Bradford.[21] The ratios of the mean enzyme specific activities (nanomoles of product formed per minute per milligram of protein) of tissue cytosols from animals treated with the inducer to those receiving vehicle only were then calculated (± SEM). The standard errors of these ratios were calculated.[18]

**Acknowledgment.** We are grateful for generous financial support from the National Cancer Institute, Department of Health and Human Services (PO1 CA 44530), and the American Institute for Cancer Research. Y.Z. is a Fellow of the Cancer Research Foundation of America. We thank Dr. N. N. Murthy (JHU, Chemistry Department) for the X-ray structure determination of 23 and Dr. G. F. Spencer (USDA, National Center for Agricultural Utilization Research, Peoria, IL) for a gift of sulforaphene.

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*Proc. Natl. Acad. Sci. USA*
Vol. 89, pp. 2399–2403, March 1992
Medical Sciences

# A major inducer of anticarcinogenic protective enzymes from broccoli: Isolation and elucidation of structure

(chemoprotection/enzyme induction/isothiocyanates/sulforaphane/quinone reductase)

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**ABSTRACT** **Consumption of vegetables, especially crucifers, reduces the risk of developing cancer. Although the mechanisms of this protection are unclear, feeding of vegetables induces enzymes of xenobiotic metabolism and thereby accelerates the metabolic disposal of xenobiotics. Induction of phase II detoxication enzymes, such as quinone reductase [NAD(P)H:(quinone-acceptor) oxidoreductase, EC 1.6.99.2] and glutathione *S*-transferases (EC 2.5.1.18) in rodent tissues affords protection against carcinogens and other toxic electrophiles. To determine whether enzyme induction is responsible for the protective properties of vegetables in humans requires isolation of enzyme inducers from these sources. By monitoring quinone reductase induction in cultured murine hepatoma cells as the biological assay, we have isolated and identified (−)-1-isothiocyanato-(4*R*)-(methylsulfinyl)butane [$CH_3$—SO—$(CH_2)_4$—NCS, sulforaphane] as a major and very potent phase II enzyme inducer in SAGA broccoli (*Brassica oleracea italica*). Sulforaphane is a monofunctional inducer, like other anticarcinogenic isothiocyanates, and induces phase II enzymes selectively without the induction of aryl hydrocarbon receptor-dependent cytochromes P-450 (phase I enzymes). To elucidate the structural features responsible for the high inducer potency of sulforaphane, we synthesized racemic sulforaphane and analogues differing in the oxidation state of sulfur and the number of methylene groups: $CH_3$—$SO_m$—$(CH_2)_n$—NCS, where $m$ = 0, 1, or 2 and $n$ = 3, 4, or 5, and measured their inducer potencies in murine hepatoma cells. Sulforaphane is the most potent inducer, and the presence of oxygen on sulfur enhances potency. Sulforaphane and its sulfide and sulfone analogues induced both quinone reductase and glutathione transferase activities in several mouse tissues. The induction of detoxication enzymes by sulforaphane may be a significant component of the anticarcinogenic action of broccoli.**

Individuals who consume large amounts of green and yellow vegetables have a lower risk of developing cancer (1–3). Feeding of such vegetables to rodents also protects against chemical carcinogenesis (4, 5), and it results in the induction in many tissues of phase II[§] enzymes—e.g., quinone reductase [QR; NAD(P)H:(quinone-acceptor) oxidoreductase, EC 1.6.99.2] and glutathione *S*-transferases (EC 2.5.1.18) (11–17). Although much evidence suggests that induction of these enzymes is a major mechanism responsible for this protection (18–20), the precise role of enzyme induction in protection of humans requires clarification. The preceding report (21) shows that measurement of QR activity in Hepa 1c1c7 murine hepatoma cells provides a rapid, reliable, and convenient index of phase II enzyme inducer activity in vegetables. Using this assay (21–24), we found that cruciferous vegetables (broccoli, cauliflower, mustard, cress, brussels sprouts) were a rich source of inducer activity. We chose to investigate broccoli (*Brassica oleracea italica*) specifically because it is consumed in substantial quantities by Western societies and has been shown to contain abundant phase II enzyme inducer activity (21). In this paper we describe the isolation and identification of a potent major phase II enzyme inducer from broccoli.

## MATERIALS AND METHODS

**Source of Vegetables and Preparation of Extracts.** SAGA broccoli was grown by Andrew Ayer (Maine Packers, Caribou, ME). SAGA is synonymous with Mariner broccoli (Petoseed, Arroyo Grande, CA) and was adapted for growing in Maine by Smith, Ayer, Goughan, and Arrow. The broccoli was harvested when ripe, frozen immediately, shipped to our laboratory in dry ice, and stored at −20°C until processed.

For preliminary survey of inducer activity in broccoli samples, florets were homogenized with 2 vol of water at 4°C, and the resultant soups were lyophilized to give powders, which were stored at −20°C. Portions (400 mg) of these powders were extracted for 6 hr with 14 ml of acetonitrile in glass-stoppered vessels on a wrist-action shaker at 4°C. The extracts were filtered through a sintered glass funnel and evaporated to dryness in a rotating evaporator (<40°C). The residues were dissolved or suspended in 100 μl of dimethyl formamide and assayed for inducer activity.

**Assay of Inducer Activity.** Inducer potency for QR was measured in Hepa 1c1c7 murine hepatoma cells grown in 96-well microtiter plates (21, 24). The cells (10,000 per well) were grown for 24 hr and then exposed to inducer for 48 hr. Usually 20 μl of the solutions to be assayed (in acetonitrile or dimethyl formamide) was added to 10.0 ml of medium and 2-fold serial dilutions were used for the microtiter plates. The final organic solvent concentration was always less than 0.2% by volume. One unit of inducer activity is defined as the amount that when added to a single microtiter well (containing 150 μl of medium) doubles the QR specific activity. The inducer potency of compounds of known structure has been determined in the above system also, and it is expressed as

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Abbreviations: QR, quinone reductase [NAD(P)H:(quinone-acceptor) oxidoreductase, EC 1.6.99.2]; CD value, the concentration of a compound required to double the quinone reductase specific activity in Hepa 1c1c7 murine hepatoma cells.

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§Enzymes of xenobiotic metabolism belong to two families (6): (*i*) phase I enzymes (e.g., cytochromes P-450), which functionalize compounds, usually by oxidation or reduction; although their primary role is to detoxify xenobiotics, several cytochromes P-450 can activate procarcinogens to highly reactive ultimate carcinogens (7); and (*ii*) phase II enzymes, which conjugate functionalized products with endogenous ligands (e.g., glutathione, glucuronic acid, sulfate) and play primarily a detoxication role (8). QR is considered a phase II enzyme because it serves protective functions (9), is induced coordinately with other phase II enzymes, and is regulated by enhancer elements similar to those that regulate glutathione transferases (10).

the concentration required to double (CD value) the QR activity.

The inductions of QR and glutathione transferase activities in mouse organs were studied according to a standard protocol (25).

**Synthesis of Compounds.** (*R,S*)-Sulforaphane (CAS 4478-93-7) was prepared according to Schmid and Karrer (26) except that gaseous thiomethanol was replaced by sodium thiomethoxide. The sulfide analogues, $CH_3$—S—$(CH_2)_n$—NCS, where *n* is 4 [erucin (CAS 4430-36-8)] or 5 [berteroin (CAS 4430-42-6)] were prepared as described (27), and the three-carbon analogue [iberverin (CAS 505-79-3)] was prepared from phthalimidopropyl bromide (26). IR spectra of all three sulfide analogues showed strong absorptions near 2150 $cm^{-1}$, characteristic of isothiocyanates. $^1H$ NMR spectra of these compounds show sharp singlets at $\delta$ 2.10 ppm ($CH_3$—S group). The sulfoxide analogues where *n* is 3 [iberin (CAS 505-44-2)] or 5 [alyssin (CAS 646-23-1)] were prepared by the same method as sulforaphane. IR spectra of these compounds showed strong absorptions near 2100 $cm^{-1}$, assigned to the —NCS group. $^1H$ NMR spectra also showed sharp singlets around $\delta$ 2.5 ppm, consistent with the presence of the $CH_3$—SO group. The sulfone analogues, $CH_3$—$SO_2$—$(CH_2)_n$—NCS, where *n* is 3 [cheirolin (CAS 505-34-0)], 4 [erysolin (CAS 504-84-7)], or 5 (unreported) were prepared by known methods (28). $^1H$ NMR ($\delta \approx 2.9$ ppm, for $CH_3$—$SO_2$—) and IR spectra of these compounds were consistent with the structures. Every analogue except 1-isothiocyanato-5-methylsulfonylpentane [$CH_3$—$SO_2$—$(CH_2)_5$—NCS] has been isolated from plants (29).

## RESULTS

**Isolation of Inducer Activity.** We selected SAGA broccoli for study because acetonitrile extracts of lyophilized homogenates of this variety were especially rich in inducer activity (62,500 units per g) in comparison with other vegetables (21). Fractionation of acetonitrile extracts of SAGA broccoli by preparative reverse-phase HPLC (Fig. 1) with a water/methanol solvent gradient resulted in recovery of 70–90% of the applied inducer activity in the chromatographic fractions. Surprisingly, the majority (about 65–80% in several chromatographies) of the recovered activity was associated with a single and relatively sharp peak [fractions 18–23; eluted at 64–71% (vol/vol) methanol]. This HPLC procedure was therefore adopted as the first step of the larger-scale isolation of inducer activity.

Lyophilized SAGA broccoli was extracted three times with acetonitrile (35 ml/g) for 6 hr each at 4°C. The pooled extracts were filtered and evaporated to dryness under reduced pressure on a rotating evaporator (<40°C). About 1 g of residue from 640 g of fresh broccoli (64 g of lyophilized powder) contained $3.6 \times 10^6$ units of inducer activity. The residue was mixed thoroughly with 120 ml of methanol/water (25/75, vol/vol) and the insoluble fraction was discarded. Although not all of the residue obtained from the extraction was soluble in aqueous methanol, the solvent partition procedure resulted in substantial purification without significant loss of inducer activity. Portions of the extract were dried in a vacuum centrifuge and dissolved in small volumes of dimethyl formamide (0.75–1.0 ml per 50 mg of residue), and 50-mg portions were subjected to HPLC (nine runs) as described in the legend of Fig. 1. Fractions 18–23 from all runs were pooled, evaporated to dryness, applied in acetonitrile to five preparative silica TLC plates (100 × 200 × 0.25 mm), and developed with acetonitrile, which was run to the top of each plate three times. Four major fluorescence-quenching components were resolved, and nearly all (99%) of the inducer activity migrated at $R_f$ 0.4. The active bands were eluted with acetonitrile, pooled, and fractionated by two runs on a second preparative reverse-phase HPLC in a water/

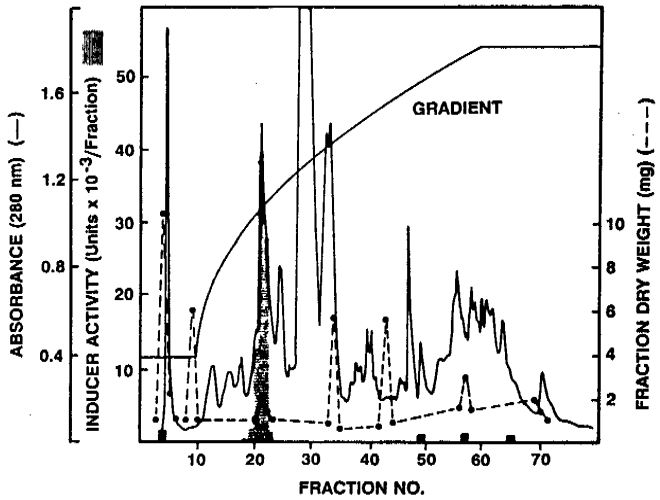
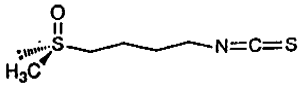



FIG. 1. Reverse-phase HPLC of acetonitrile extract of SAGA broccoli showing the distribution of absorbance at 280 nm, total inducer activity (units per fraction), and dry weight of each fraction. Lyophilized SAGA broccoli floret powder (16 g) was extracted three times (for 6 hr each) with 560-ml portions of acetonitrile on a shaker at 4°C. The extracts were filtered and evaporated to dryness on a rotating evaporator (<40°C). The residue (202 mg) was suspended in 3.0 ml of methanol and filtered successively through 0.45- and 0.22-μm porosity filters. The insoluble material was discarded. The filtrate was assayed for total inducer activity, and a 0.75-ml (50.5-mg) aliquot of the methanol extract was subjected to HPLC on a reverse-phase preparative column (Whatman; Partisil 10 ODS-2; 50 × 1.0 cm) equilibrated with methanol/water (30/70, vol/vol), eluted at a rate of 3.0 ml/min, and 6.0-ml fractions were collected. Elution solvent: 30 ml of initial solvent, followed by 330 ml of a convex gradient (Waters Gradient program 5) to 100% methanol, and then by 90 ml of 100% methanol. The fractions were evaporated on a vacuum centrifuge (Savant Speed-Vac Concentrator), and the residues were weighed, redissolved in 0.1 ml of dimethyl formamide, and assayed for inducer activity. The activity applied (0.75 ml = 104,000 units) was recovered principally in fractions 18–23 (84,600 units, 81%), and minor amounts of activity were found in fractions 4, 49, 57, and 65. The total recovery of inducer activity in all fractions was 90% of that applied to the column.

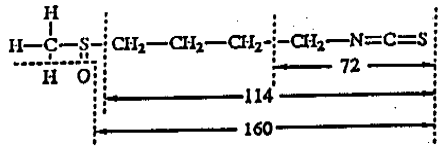
acetonitrile gradient (Fig. 2). Ultraviolet absorption and inducer activity were eluted in a sharp coincident peak (at 66% acetonitrile) that contained all of the activity applied to the column. Evaporation (<40°C) of the active fractions gave 8.9 mg of a slightly yellow liquid, which contained 558,000 inducer units (overall yield 15%) and migrated as a single band on TLC.

**Identification of Inducer.** The identity of the inducer was established by spectroscopic methods and confirmed by chemical synthesis. It is (−)-1-isothiocyanato-(4*R*)-(methylsulfinyl)butane, known as sulforaphane or sulphoraphane (CAS 4478-93-7):



Sulforaphane has been synthesized (26) and isolated from leaves of hoary cress (30) and from other plants (31), and the absolute configuration has been assigned (32). The closely related olefin sulforaphene [4-isothiocyanato-(1*R*)-(methylsulfinyl)-1-(*E*)-butene (CAS 2404-46-8)] has been isolated from radish seeds and other plants (33, 34) and has also been synthesized (35, 36).

The following evidence establishes that (*R*)-sulforaphane is the inducer isolated from broccoli. UV spectrum ($H_2O$): $\lambda_{max}$ 238 nm, $\varepsilon_{238}$ 910 $M^{-1}\cdot cm^{-1}$; addition of NaOH (0.1 M) blue-shifted ($\lambda_{max}$ 226 nm) and intensified ($\varepsilon_{226}$ 15,300 $M^{-1}\cdot cm^{-1}$) this absorption band, consistent with the behavior of isothiocyanates (37): IR (Fourier transform, neat): strong absorptions at 2179 and 2108 $cm^{-1}$ and also at 1350 $cm^{-1}$, characteristic of isothiocyanates (27). $^1H$ NMR (400 MHz, $C^2HCl_3$): δ 3.60 (t, 2H, $J$ = 6.1 Hz, $—CH_2—NCS$), 2.80–2.66 (m, 2H, $—CH_2—SO—$), 2.60 (s, 3H, $CH_3—SO—$), and 1.99–1.86 ppm (m, 4H, $—CH_2CH_2—$). $^{13}C$ NMR (400 MHz, $C^2HCl_3$): δ 53.5, 44.6, 38.7, 29.0, and 20.1 ppm. Mass spectrometry (fast atom bombardment; thioglycerol matrix) gave prominent peaks at 178 $(M + H)^+$ and 355 $(M_2 + H)^+$. Electron impact mass spectrometry gave a small molecular ion ($M^+$) at 177, and chemical ionization mass spectrometry gave a small molecular ion $(M + H)^+$ at 178 and prominent fragment ions with masses of 160, 114, and 72, consistent with the following fragmentation:



Precise masses of molecular and fragment ions obtained by electron impact mass spectrometry were 177.0286 (calculated for $C_6H_{11}NOS_2$, 177.0283), 160.0257 (calculated for $C_6H_{10}NS_2$, 160.0255), and 71.9909 (calculated for $C_2H_2NS_1$, 71.9908). In addition, for the mass 160 fragment, the peaks at 161 (M + 1) and 162 (M + 2) were 8.43% (calculated, 8.44%) and 9.45% (calculated, 10.2%), respectively, of the parent ion. Similarly, for the mass 72 fragment, the peaks at 73 (M + 1) and 74 (M + 2) were 3.42% (calculated, 3.32%) and 5.23% (calculated, 4.44%), respectively, of the parent ion. Hence the isotope compositions corrected for the natural isotope abundance (of $^{13}C$, $^{15}N$, $^{33}S$, and $^{34}S$) were consistent with the relative intensities of the M + 1 and M + 2 ions of both fragments. The optical rotation of the isolated material was $[\alpha]_D^{23}$ −63.6° ($c$ = 0.5, $CH_2Cl_2$), thus establishing that the product is largely, if not exclusively, the (−)-(*R*) enantiomer (literature $[\alpha]_D$ −79°, −73.2°, −66°; refs. 26, 30, and 38, respectively). The spectroscopic properties of synthetic (*R,S*)-sulforaphane were identical to those of the isolated product.

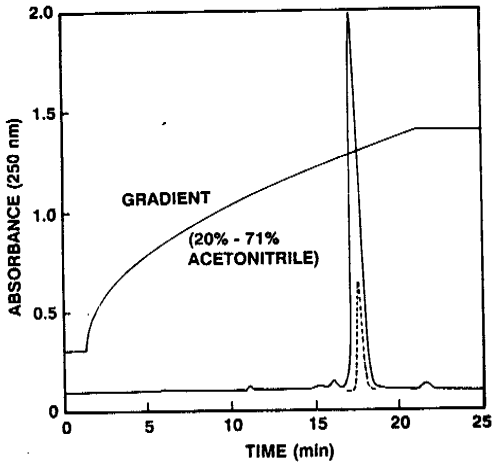



FIG. 2. Second reverse-phase preparative HPLC of enzyme inducer activity from SAGA broccoli. The active inducer bands obtained from two or three preparative silica TLC plates (see text) were combined, eluted with acetonitrile, filtered twice through 0.22-μm porosity filters, and evaporated to dryness on a vacuum centrifuge. The residue was dissolved in 0.5 ml of acetonitrile and applied to a reverse-phase preparative HPLC column (Whatman; Partisil ODS-2; 50 × 1.0 cm), which was developed with a convex gradient (Waters Gradient program 5) of acetonitrile/water from 20:80% to 71:29% (vol/vol) at a flow rate of 3.0 ml/min during a 20-min period. The eluate from 17.0 to 19.0 min was collected as a pool and assayed for inducer activity; 99% of the inducer activity was recovered in this pool. The elution position of (*R,S*)-sulforaphane is shown (---).

**Relation of Structure to Inducer Activity Among Sulforaphane Analogues.** To define the structural features of sulforaphane (chirality, state of oxidation of sulfur of the thiomethyl group, number of methylene bridging groups) important for inducer activity, we synthesized (*R,S*)-sulforaphane and the following analogues and measured their inducer potencies: $CH_3—S—(CH_2)_n—N=C=S$ ($n$ = 3, 4, or 5); $CH_3—SO—(CH_2)_n—N=C=S$ ($n$ = 3 or 5); and $CH_3—SO_2—(CH_2)_n—N=C=S$ ($n$ = 3, 4, or 5).

**Induction of QR in Murine Hepatoma Cells.** The chirality of the sulfoxide does not affect inducer potency, since isolated (*R*)-sulforaphane and synthetic (*R,S*)-sulforaphane gave closely similar CD values of 0.4–0.8 μM. Sulforaphane is therefore the most potent monofunctional (see below) inducer that has been identified (19, 20). Both (*R*)- and (*R,S*)-sulforaphane were relatively noncytotoxic: the concentrations required to depress cell growth to one-half were 18 μM.

Sulforaphane and the corresponding sulfone (erysolin) were equipotent as inducers of QR, whereas the corresponding sulfide (erucin) was about one-third as active (Table 1). Oxidation of the side-chain sulfide to sulfoxide or sulfone enhanced inducer potency, and compounds with 4 or 5 methylene groups in the bridge linking $CH_3S—$ and $—N=C=S$ were more potent than those with 3 methylene groups (Table 1).

Mutants of Hepa 1c1c7 cells defective in the Ah (aryl hydrocarbon) receptor or expression of cytochrome P-450IA1 can distinguish monofunctional inducers (which induce phase II enzymes selectively) from bifunctional in-

Table 1. Potency of induction of QR in Hepa 1c1c7 cells by sulforaphane and analogues

| Compound | CD value, μM | | |
|---|---|---|---|
| | $n = 3$ | $n = 4$ | $n = 5$ |
| $CH_3—S—(CH_2)_n—N=C=S$ | 3.5 (Iberverin) | 2.3 (Erucin) | 1.7 (Berteroin) |
| $CH_3—S—(CH_2)_n—N=C=S$ (O) | 2.4 (Iberin) | 0.4–0.8 (Sulforaphane) | 0.95 (Alyssin) |
| $CH_3—S(=O)(=O)—(CH_2)_n—N=C=S$ | 1.3 (Cheirolin) | 0.82 (Erysolin) | 0.98 |

Trivial names are given in parentheses. See Kjær (29).

ducers (which elevate both phase I and II enzymes) (39, 40). When sulforaphane was tested with the BP$^r$c1 mutant (41) (defective in transport of the liganded Ah receptor to the nucleus), and the c1 mutant (42) (which synthesizes inactive cytochrome P-450IA1), induction of QR was normal (data not shown). Sulforaphane is, therefore, like benzyl isothiocyanate, a monofunctional inducer (40) and is unlikely to elevate activities of cytochromes P-450 that could activate carcinogens.

**Induction of QR and Glutathione Transferase Activities in Mice.** When synthetic (*R,S*)-sulforaphane, erysolin, and erucin were administered to female CD-1 mice by gavage (25), induction of QR and glutathione transferase activities was observed in the cytosols of several organs (Table 2). Sulforaphane and erucin (in daily doses of 15 μmol for 5 days) raised both enzyme activities 1.6- to 3.1-fold in liver, forestomach, glandular stomach, and mucosa of proximal small intestine, and to a lesser degree in lung. The sulfone (erysolin) was more toxic, but even 5-μmol daily doses for 5 days elevated the specific activities of these enzymes in some tissues examined. We therefore conclude that sulforaphane and its analogues not only induce QR in Hepa 1c1c7 murine hepatoma cells but also induce both QR and glutathione transferase activities in a number of murine organs.

## DISCUSSION

Two considerations prompt the belief that sulforaphane is a major and probably the principal inducer of phase II enzymes present in extracts of SAGA broccoli. First, high yields of inducer activity were obtained at each step of the isolation, and even in the first HPLC (Fig. 1) more than 60% of the inducer activity was contained in a single chromatographic peak, the biological activity of which could not be subfractionated. Second, when a totally independent method of isolation of inducer activity by high-vacuum sublimation of lyophilized broccoli (5 μm Hg pressure, 60–165°C, condenser at −15°C) was used, nearly all the isolated inducer activity was found in the methanol-soluble portion of the sublimate. Moreover, on HPLC (Fig. 2) this sublimed material gave rise to only a single isothiocyanate-containing fraction, which on TLC comigrated with authentic sulforaphane and after further purification by TLC provided a high yield of sulforaphane characterized unequivocally by NMR.

The finding that the majority of the inducer activity of SAGA broccoli probably resides in a single chemical entity, an isothiocyanate, is of considerable interest. Isothiocyanates (mustard oils) and their glucosinolate precursors are widely distributed in higher plants and are especially prevalent among cruciferous vegetables (29). Sulforaphane has been identified in species of *Brassica*, *Eruca*, and *Iberis* (29, 31).

Isothiocyanates have been shown to block chemical carcinogenesis. In rats, 1-naphthyl isothiocyanate suppressed hepatoma formation by 3-methylcholanthrene, 2-acetylaminofluorene, diethylnitrosamine, *m*-toluenediamine, and azo dyes (43–46). In mice, benzyl isothiocyanate blocked the neoplastic effects of diethylnitrosamine or benzo[*a*]pyrene on lung and forestomach (47, 48), and a variety of phenylalkyl isothiocyanates reduced the pulmonary carcinogenicity of

Table 2. Induction of QR and glutathione *S*-transferase (GST) in mouse tissues by sulforaphane and analogues

| Inducer | Dose, μmol per mouse per day | Enzyme | Ratio of specific activities (treated/control) | | | | |
|---|---|---|---|---|---|---|---|
| | | | Liver | Forestomach | Glandular stomach | Proximal small intestine | Lung |
| $CH_3—S—(CH_2)_4—NCS$ Erucin | 15 | QR | 2.19 ± 0.06 | 1.64 ± 0.18* | 1.72 ± 0.11 | 3.10 ± 0.20 | 1.66 ± 0.13 |
| | | GST | 1.86 ± 0.08 | 2.51 ± 0.11 | 2.07 ± 0.08 | 3.00 ± 0.21 | 1.41 ± 0.11* |
| $CH_3—S(=O)—(CH_2)_4—NCS$ Sulforaphane | 15 | QR | 2.45 ± 0.07 | 1.70 ± 0.18* | 2.35 ± 0.06 | 2.34 ± 0.19 | 1.37 ± 0.14* |
| | | GST | 1.86 ± 0.08 | 1.98 ± 0.08 | 2.97 ± 0.08 | 2.13 ± 0.20 | 1.17 ± 0.09† |
| $CH_3—S(=O)(=O)—(CH_2)_4—NCS$ Erysolin | 5 | QR | 1.62 ± 0.09 | 1.05 ± 0.21† | 1.57 ± 0.08† | 1.22 ± 0.20† | 1.00 ± 0.11† |
| | | GST | 1.08 ± 0.11† | 1.45 ± 0.15† | 1.94 ± 0.10† | 0.87 ± 0.20† | 1.09 ± 0.13† |

The compounds were administered to 6-week-old female CD-1 mice (4 or 5 mice per group) by gavage in indicated single daily doses in 0.1 ml of Emulphor EL 620P (GAF, Linden, NJ) for 5 days. Cytosols were prepared from the tissues 24 hr after the last treatment and assayed for enzyme activities (glutathione *S*-transferase was measured with 1-chloro-2,4-dinitrobenzene). The specific activities (nmol·min$^{-1}$·mg$^{-1}$ ± SEM) of organs of vehicle-treated control mice were as follows. Liver: QR, 47 ± 0.70; GST, 1014 ± 69. Forestomach: QR, 1038 ± 155; GST, 1182 ± 74. Glandular stomach: QR, 3274 ± 85; GST, 1092 ± 81. Small intestine: QR, 664 ± 119; GST, 1372 ± 266. Lung: QR, 54 ± 5.8; GST, 439 ± 34. Data are presented as mean ± SEM. All ratios were significantly different from 1.0 with $P < 0.01$, except for *, $P < 0.05$, and †, $P > 0.05$.

the tobacco-derived carcinogenic nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (49, 50). The anticarcinogenic effects of previously studied isothiocyanates may be related to their capacity to induce phase II enzymes, which are involved in the metabolism of carcinogens (51–57).

It will be important to establish whether the alterations of drug metabolism observed in humans and rodents after the ingestion of cruciferous vegetables (58, 59) can be ascribed to their content of sulforaphane. The finding that this isothiocyanate is a major monofunctional inducer of phase II enzymes in broccoli also provides the possibility of clarifying the relationship between enzyme induction and chemoprotection.

We thank Andrew Ayer for supplying generous quantities of SAGA broccoli. These studies were supported by grants from the National Cancer Institute (PO1 CA 44530) and from the American Institute for Cancer Research. All animal experiments were in compliance with National Institutes of Health guidelines (60) and were approved by the Committee on Animal Care of Johns Hopkins University.

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